PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 05 June 2000 (05.06.00)	
International application No. PCT/US99/25552	**Applicant's or agent's file reference** 11613.32WO01
International filing date (day/month/year) 29 October 1999 (29.10.99)	**Priority date** (day/month/year) 31 October 1998 (31.10.98)
Applicant KASHMIRI, Syed, V., S. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 May 2000 (04.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
11613.32W001		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 25552	29/10/1999	31/10/1998
Applicant		
THE GOVERNEMENT OF THE UNITED STATES OF AMERICA		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 99/ 25552

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 42 is directed to a method of treatment of the human/animal body and claims 43-47 (all partially) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]US 99/25552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N15/62 C12N5/10 C07K16/30 C07K16/46
A61K51/10 A61P35/00 G01N33/574 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y SHA ET AL: "A heavy-chain grafted antibody that recognizes the tumor-associated TAG72 antigen" CANCER BIOTHERAPY, vol. 9, no. 4, 1 January 1994 (1994-01-01), pages 341-349, XP002079337 abstract page 342, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 346, left-hand column, paragraph 2 -page 347, right-hand column, paragraph 1 ---	1-47
X	WO 97 26010 A (SMITHKLINE BEECHAM CORP., USA;UNIVERSITY OF VERMONT AND STATE AGRICULT) 24 July 1997 (1997-07-24) page 9, line 28 -page 10, line 10 page 21, line 25 -page 22, line 13 ---	1,2,4,6, 7,9, 36-41
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: "Complementarity determining region residues aspartic acid at H55, serine at H95 and tyrosines at H97 and L96 play important roles in the B72.3 antibody-TAG72 antigen interaction." retrieved from STN Database accession no. 97015918 XP002134981 abstract & PROTEIN ENGINEERING, (1996 JUN) 9 (6) 539-43. , ---	23,36-47
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: "The tyrosine residue at position 97 in the VH CDR3 region of a mouse/human chimeric anti-colorectal carcinoma antibody contributes hydrogen bonding to the TAG72 antigen." retrieved from STN Database accession no. 95102752 XP002134982 abstract & CANCER BIOTHERAPY, (1993 FALL) 8 (3) 253-62. , ---	23,36-47
A	WO 96 13594 A (US HEALTH) 9 May 1996 (1996-05-09) page 24, line 9 -page 26, line 3 examples 13,17,18 ---	1-47
P,A	WO 99 43816 A (ARMOUR KATHRYN ;CARR FRANK J (GB); HARRIS WILLIAM J (GB); TEMPEST) 2 September 1999 (1999-09-02) example 1 claims ---	1-47
T	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US IWAHASHI M ET AL: "CDR substitutions of a humanized monoclonal antibody (CC49): contributions of individual CDRs to antigen binding and immunogenicity." retrieved from STN Database accession no. 2000162136 XP002134983 abstract & MOLECULAR IMMUNOLOGY, (1999 OCT-NOV) 36 (15-16) 1079-91. , --- -/--	1-47

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	TAMURA M ET AL: "Structural correlates of an anticarcinoma antibody: identification of specificity-determining residues (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only." JOURNAL OF IMMUNOLOGY, (2000 FEB 1) 164 (3) 1432-41. , XP000901556 the whole document	1-47

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/25552

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9726010	A	24-07-1997	AU	706397 B	17-06-1999
			AU	1830897 A	11-08-1997
			CN	1213312 A	07-04-1999
			HU	9900396 A	28-05-1999
			NO	983284 A	16-09-1998
			PL	327929 A	04-01-1999
			US	6005091 A	21-12-1999
			ZA	9700347 A	06-10-1998
WO 9613594	A	09-05-1996	US	5889157 A	30-03-1999
			US	5981726 A	09-11-1999
			US	5608039 A	04-03-1997
			AU	4135596 A	23-05-1996
			CA	2203236 A	09-05-1996
			EP	0796334 A	24-09-1997
			JP	10508202 T	18-08-1998
			US	5990296 A	23-11-1999
WO 9943816	A	02-09-1999	AU	6439398 A	15-09-1999

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/25552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N15/62 C12N5/10 C07K16/30 C07K16/46
A61K51/10 A61P35/00 G01N33/574 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y SHA ET AL: "A heavy-chain grafted antibody that recognizes the tumor-associated TAG72 antigen" CANCER BIOTHERAPY, vol. 9, no. 4, 1 January 1994 (1994-01-01), pages 341-349, XP002079337 abstract page 342, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 346, left-hand column, paragraph 2 -page 347, right-hand column, paragraph 1	1-47
X	WO 97 26010 A (SMITHKLINE BEECHAM CORP., USA;UNIVERSITY OF VERMONT AND STATE AGRICULT) 24 July 1997 (1997-07-24) page 9, line 28 -page 10, line 10 page 21, line 25 -page 22, line 13	1,2,4,6, 7,9, 36-41

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

Form PCT/ISA/210 (second sheet) (July 1992)

Inter al Application No
PCT/US 99/25552

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	TAMURA M ET AL: "Structural correlates of an anticarcinoma antibody: identification of specificity-determining residues (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only." JOURNAL OF IMMUNOLOGY, (2000 FEB 1) 164 (3) 1432-41. , XP000901556 the whole document	1-47

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726010 A	24-07-1997	AU 706397 B	17-06-1999
		AU 1830897 A	11-08-1997
		CN 1213312 A	07-04-1999
		HU 9900396 A	28-05-1999
		NO 983284 A	16-09-1998
		PL 327929 A	04-01-1999
		US 6005091 A	21-12-1999
		ZA 9700347 A	06-10-1998
WO 9613594 A	09-05-1996	US 5889157 A	30-03-1999
		US 5981726 A	09-11-1999
		US 5608039 A	04-03-1997
		AU 4135596 A	23-05-1996
		CA 2203236 A	09-05-1996
		EP 0796334 A	24-09-1997
		JP 10508202 T	18-08-1998
		US 5990296 A	23-11-1999
WO 9943816 A	02-09-1999	AU 6439398 A	15-09-1999

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

FEB 16 2000

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

DAIGNAULT, Ronald, A.
Merchant & Gould P.C.
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55402-4131
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 31 January 2000 (31.01.00)	
Applicant's or agent's file reference 11613.32WO01	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/25552	International filing date (day/month/year) 29 October 1999 (29.10.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 31 October 1998 (31.10.98)

Applicant

THE GOVERNEMENT OF THE UNITED STATES OF AMERICA as represented by THE ~~SECRETARY, DEPARTMENT OF HEALT et al~~

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
31 Octo 1998 (31.10.98)	60/106,534	US	24 Janu 2000 (24.01.00)
02 Nove 1998 (02.11.98)	60/106,757	US	24 Janu 2000 (24.01.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Taïeb Akremi

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
11613.32WO01		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/25552	29/10/1999	31/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/85		
Applicant THE GOVERNEMENT OF THE UNITED STATES OF AMERICA		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/05/2000	15.12.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Novak, S Telephone No. +49 89 2399 8930

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/25552

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-46 as originally filed

Claims, No.:

1-47 as originally filed

Drawings, sheets:

1/23-23/23 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 47
	No:	Claims	
Inventive step (IS)	Yes:	Claims	10
	No:	Claims	1 - 9, 11 - 47
Industrial applicability (IA)	Yes:	Claims	1 - 41
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: Y SHA ET AL: CANCER BIOTHERAPY, vol. 9, no. 4, 1 January 1994, pages 341-349
D2: WO 97 26010 A, 24 July 1997
D3: DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: retrieved from STN Database accession no. 95102752 XP002134982 & CANCER BIOTHERAPY, (1993 FALL) 8 (3) 253-62.

ad V.

1. Novelty (Article 33(2) PCT)

1.1. The present application is drawn to mouse-human chimeric variants of CC49 monoclonal antibodies with minimal murine content.
Also encompassed are biotechnological methods of making the variants and therapeutic methods of using the variants.

1.2. This subject-matter is not described in any prior art document, and therefore regarded to fulfill the requirements of Article 33(2) PCT.

2. Inventive Step (Article 33(3) PCT)

2.1. D1 is considered to represent the closest prior art.
This document describes a heavy-chain grafted antibody that recognizes the tumour-associated TAG72 antigen.
This is achieved by the transplantation of CDRs from the murine Vh of the ccM4 antibody into FRs of the human myeloma protein NEWM. This humanized antibody retains its binding reactivity for the TAG72 antigen, though less than the original chimeric antibody.
These results indicate that the murine anti TAG72 specificity can be grafted to human immunoglobulin, and that the choice of the human immunoglobulin framework has importance for the maintenance of the immunoreactivity (see abstract).

2.2. The present application differs therefrom inasmuch claimed humanized anti-TAG-72 antibodies comprise either light chain CD regions of human origin, or particular substitutions of heavy chain CD regions not specifically mentioned in the prior art document.

2.3. The problem to be solved by the present application may be regarded therefore to provide additional anti-TAG-72 humanized antibodies.

2.4. In order to solve the problem to which the application refers the skilled person would take into account the teaching of D2 and/or D3.

D2 describes the principle and theoretical background for "engineered antibodies", i.e., a full-length synthetic antibody in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope (see page 9).
It is pointed out in this document that "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulin. In addition, framework support residues may be altered to preserve binding affinity (see page 10).

D3 is concerned with the tyrosine residue at position 97 in the VH CDR3 region of a mouse/human chimeric anti-TAG72 antibody. A single amino acid substitution at this position resulted in approximate 18-fold lower binding affinity, which suggests that the tyrosine residue at position 97 is in a contact position in the antibody/antigen interaction.

2.5. It follows that motivated by this knowledge the skilled person had the necessary background information in order to apply the teaching of D2 to an anti-TAG-72 antibody as described in D1, and thus to arrive at antibodies claimed in the present application. For the substitution of particular amino acids, such as Tyr97, the results described in D3 give the necessary motivation with reasonable expectation of success.

Therefore, no inventive step can be acknowledged for the subject-matter of claims 1 - 9, and 11- 35.
The same is true for compositions comprising such antibodies, or nucleic acids encoding said immunoglobulins, respectively methods for treating cancer using such antibodies.
Such subject-matter is regarded to represent obvious applications, or uses of said immunoglobulins, which come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can be readily foreseen.
Consequently, the subject-matter of claims 36 - 47 is not regarded to fulfill the requirements of Article 33(3) PCT.
The subject-matter of claim 10 however, which is concerned with the CC49 anti-TAG-72 antibody, is considered to involve an inventive step.

3. Industrial applicability

3.1. Claims 42 - 47 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

ad VIII.

4. Clarity (Article 6 PCT)

4.1. It is clear from the description on page 4 that the following feature is essential to the definition of the invention:

(1) "variants of CC49"

Since independent claims 1, 11, 23, 34, and 35 do not contain this feature they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

4.2. Furthermore, claims 11, 23, 34, and 35 are concerned with antibodies which have been substituted at particular amino acid residues, e.g. position 60, 61, 62, or 64 in H-CDR2.
Such a description appears completely confusing, and unclear in the light that no specific antibody has been pointed out to which these positions would refer.

4.3. As a general rule, the area defined by the claims must be as precise as the invention allows.
It is emphasised that claims must be clear on their own and that they must state the technical features which are necessary for the definition of the claimed subject-matter.
Consequently, dependent claims 2 - 9, 12 - 22, and 24 - 33 do also not meet the requirements of Article 6 PCT.

4.4. It follows from 4.1. - 4.3. that claims 1 - 9, and 11 - 35 are not allowable.
The above objections can only be met by restricting the scope to those antibodies that can be shown to solve the problem posed, i.e. the antibodies having the essential structural feature (variants of CC49, having a defined amino acid structure).

Table 1: Patient Characteristics

Dose Level	Patient	Age	Sex	Tumor	Dose[a] mCi	Dose[a] mg MAb
10 mCi/m^2	DS	52	F	Breast	16.0	20
	LW	45	F	Breast	19.0	20
	JJ	61	F	Breast	17.2	20
25 mCi/m^2	DG	45	F	Breast	41.0	20
	LJ	45	F	Breast	40.3	20
	JM	42	F	Breast	45.4	20
15 mCi/m^2	JG	61	M	Colon	29.8	44
	RW	46	F	Lung	24.2	20
	TD	50	M	Colon	31.5	47
	EA[b]	53	F	Colon	24.2	20
	CP[b]	53	F	Colon	26.0	20
	LQ[b]	45	F	Colon	29.7	20

[a] Patients were administered ^{177}Lu–PA–DOTA–CC49 by intravenous injection.
[b] Patient received new formulation of ^{177}Lu–PA–DOTA–CC49 that was labeled using a modification of the method described by Mulligan et al. (1995), Clin. Cancer Res. 1:1447–1454.

PA–DOTA was conjugated to human serum albumin (HSA), radiolabeled with Na^{125}I, incubated with the patient sera and analyzed for immune complex formation by size–exclusion HPLC. None of the sera showed detectable reactivity with the PA–DOTA–HSA conjugate (Data not shown).

Determination of Patient Humoral Response

The sera from the twelve patients was evaluated for the presence of human anti–murine antibodies (HAMA) in response to MAb CC49 using high performance liquid chromatograph (HPLC) as described by Mulligan et al. (1996) Clin. Cancer Res., 1:1447–1454. The analysis was performed by adding about 500,000 cpm (0.4μCi) of ^{125}I–BL–3 to 50 μl of patient sera. Following a 60 minute incubation at 37°C, 25 μl of the mixture was applied to a size–exclusion column (TSK 3000SW; TosoHaas, Montgomeryville, PA) equilibrated in 67 mM sodium phosphate (pH 6.8) containing 100 mM KCl. The sera samples were eluted at a flow rate of 0.5 ml/min. The protein was detected by absorbance at 280 nm and the radioactivity was measured using a flow–through γ–scintillation counter (Model 170, Beckman Instruments, Inc., Berkeley, CA). The presence of HAMA was indicated by a shift in the elution profile of the ^{125}I–BL–3 because the formation of immune complexes with the radiolabeled BL–3 results in a shorter retention time. The patients' pre–study sera, normal human sera and phosphate buffered saline with ^{125}I–BL–3 were used as controls. A patient with a known HAMA response from a previous study

(Colcher et al. (1990), J. Nucl. Med., 31:1133–1142) served as a positive control. The patients' sera were demonstrated to have antibodies against the variable region of the murine CC49.

Figure 19 shows an HPLC analysis of patient HAMA following intravenous injection of ^{177}Lu–CC49. Serum samples from LQ were analyzed for the presence of HAMA at various timepoints before and after injection with 20 mg of ^{177}Lu–labeled CC49. Pre–study sera (A), sera collected at 7 days (B), 3 weeks (C), and 6 weeks (D) were mixed with ^{125}I–BL–3 and applied to a size exclusion column. Reduction in retention time of the radiolabeled BL–3 as compared to migration of the ^{125}I–BL–3 in buffer (E) were indicative of immune complex formation and therefore the presence of HAMA.

Lack of complex formation is evident (FIG. 19A) when the pre–study sera of Patient LQ is incubated with the ^{125}I–BL–3. All of the radioactivity is associated with the peak at about 18.5 minutes, the same retention time for ^{125}I–BL–3 in buffer (FIG. 19E). Complex formation is also absent when the sera collected at seven days is incubated with ^{125}I–BL–3 (FIG. 19B). With sera collected at 3 weeks (FIG. 19C), however, there is an indication of complex formation (46%) with the appearance of two peaks with a shorter retention time (i.e., 14 and 16 minutes). The peaks at a shorter retention time indicate the development of a higher molecular weight species in the sera. At 6 weeks (FIG. 19D), the HAMA response has increased, the amount of radioactivity bound in complexes is now 66%.

Figure 20 shows an HPLC analysis of patients' humoral response to the variable region of MAb CC49. The percent complex formation has been plotted versus time for (solid lines) patients DS (O), LW (□), JJ (Δ), DG (●), LJ (■), TD(▲); (dotted lines) JG (O), RW (□), JM (Δ), EA (●), CP (■), LQ (▲);

At one week, none of the patients showed a detectable response against the HuCC49 (FIG. 20). At 3 weeks, sera from nine of the twelve patients (75%) appears to contain antibody against the variable region of CC49 with one patient having a notably higher response than the others. For the eleven patients evaluated at six weeks, only two patients did not elicit a human antivariable region antibody response (HAVRA) against CC49, i.e., 9 of 11 evaluable patients (82%) had antibody against the variable region of the murine MAb CC49.

Three patterns of HAMA–HAVRA response are evident. The patterns of the HAMA and HAVRA responses elicited in each of the patients were very similar, differing only in the apparent level of antibody. Patients DG, LW, LQ and CP

developed HAVRA simultaneously with HAMA. Patients DS and JM appear to have a strong HAVRA, while HAMA response is modest. While in patients TD, JG, and EA, the HAVRA level is lower than HAMA at 3 weeks, followed by HAMA and HAVRA attaining high levels at later timepoints. In no patient was there a HAVRA response without the development of HAMA..

The HAMA results for the twelve patients are summarized below in Table 2.

Table 2: HPLC Analysis of Patients' Anti-mouse immunoglobulin response after i.v. injection of ^{177}Lu-CC49

	Days Post-Injection of ^{177}Lu-CC49			
Patient	**7**	**21**	**42**	**56**
DS	0[a]	1	16	27
LW	3	6	81	NA
JJ	0	12	3	4
DG	0	24	84	NA
LJ	0	42	NA	NA
JM	0	8	47	NA
JG	4	83	83	NA
RW	0	1	2	NA
TD	0	95	100	NA
EA	0	27	100	100
CP3	0	33	27	NA
LQ	0	46	66	100

[a] The values are the percent of ^{125}I-BL-3 detected in complexes after a brief incubation with the patient sera and resolved by size-exclusion chromatography. The timepoints of each patient are background corrected using the patients' pre-study sera.

The patterns of the HAMA responses are varied and are consistent with previous findings by Colcher et al. (1990), J. Nucl. Med. 31:1133–1142. Ten out of the twelve patients (83%) demonstrate a HAMA response at 3 weeks following a single intravenous injection of 20 mg ^{177}Lu–labeled CC49, two patients (LW and JG) have minimal responses evident at 7 days with complexes of 3% and 4%, respectively. One patient (RW) may be considered a nonresponder. Some of the patients show an escalating HAMA response, while others plateau. Yet another (JJ) peaks at 3 weeks, followed by an apparent decrease in the HAMA level. Overall, at 3 weeks, 8 of 12 patients (57%) at and 6 weeks, 9 of 11 (82%) were HAMA positive.

Specificity of Patient Response

The specificity of the patients' antibody response to CC49 was assessed using ^{125}I–labeled HuCC49 and HuCC49 CDR–replacement variants to determine whether or not any of the responses were directed against the variable region of CC49. To accomplish this, the HPLC methodology was employed using ^{125}I–HuCC49 as the probe (See, Kashmiri et al. (1995), Hybridoma, 14:461–473).

To eliminate the artifactual influence of TAG–72 in the HPLC analysis for anti–CC49 antibody responses found in the patient's serum, immunoadsorbents were prepared as reported by Ferroni et al. (1992) J. Clin. Lab. Analysis, 4:465–473. For the purpose of these studies, purified MAb CC92 was coupled to Reacti–gel (HW65F, Pierce) according to the method of Heam et al. (1979), J. Chromatog., 185:463–470. MAb CC92 is a second–generation monoclonal antibody that reacts with TAG–72, but with an epitope distinct from the one recognized by CC49.

Before probing the patients' sera with the ^{125}I–HuCC49, removal of HAMA and circulating TAG–72 were confirmed using ^{125}I–BL–3 and ^{125}I–B72.3, respectively (data not shown). MAb B72.3 is an anti–TAG–72 MAb that has been shown to form complexes with TAG–72 in patient sera (Colcher et al. (1990), J. Nucl. Med., 31:1133–1142).

In the competition assay, 5 μg of the cold competitor (either purified HuCC49 or one of its variants) was added to a mixture of patient sera (collected 8 weeks post–i.v. injection with ^{177}Lu–CC49) and ^{125}I–HuCC49 and then analyzed by size–exclusion chromatography for the absence or presence of complexes. The percent inhibition of complex formation was calculated. If the variant competed with the ^{125}I–labeled MAb, and complex formation was inhibited, then the variant

still contained the immunodominant CDR. If the variant failed to inhibit complex formation, then the CDR that is no longer present in the variant is recognized by the patient and hence it is an immunogenic CDR. An example of this assay (using serum from patient LQ) is shown in FIG. 21. Panel A is the profile of the ^{125}HuCC49 in buffer only. Panel B, is the profile showing complex formation (42.9%) resulting from patient sera (LQ) incubated with ^{125}I–HuCC49. When HuCC49 is added as a competitor, there is competition for the ^{125}I–HuCC49 and a loss or absence of complexes is observed (Panel C). The same is true of a variant which still contains an immunogenic CDR (e.g., light chain CDR2 as the competitor) (Panel D). In contrast, there is either a partial (Panel F) or total retention of the complexes (Panel E), when light chain CDR1 or CDR3 variants, respectively, are the competitors.

The results are very striking, see Table 3.

Table 3: HPLC Analysis of Patient Reactivity to CDR–Replacement variants of HuCC49[a]

Competitor		Patient					
	CDR[b]	DS	DG	JG	EA	CP	LQ
None	—	33.5[c]	46.2	24.5	56.8	32.2	42.9
HuCC49	—	0	0	2.6	0.5	1.5	3.0
Hu IgG	—	46.4	59.0	25.1	63.6	ND	54.1
Light Chain	1	16.0	12.2	9.8	10.1	16.9	14.3
	2	2.7	3.4	2.7	4.4	3.0	2.4
	3	34.8	48.2	22.4	37.6	33.5	46.7
	1,2	24.6	24.5	12.6	19.4	15.7	20.2
Heavy Chain	1	10.2	3.9	3.3	7.0	5.8	3.5
	2	32.7	32.5	12.7	24.7	29.7	36.6
	3	7.3	5.1	3.7	8.2	6.7	4.6

[a] The sera from patients injected with ^{177}Lu–CC49 were tested for reactivity with variants of HuCC49 in which individual CDRs had been substituted with human sequences in both the heavy and light chains of HuCC49. Five μg of the purified CDR–replacement variants were added to a mixture of ^{125}I–HuCC49 and the patient sera and then analyzed for the presence or absence of immune complex formation.
[b] The number indicates which CDR in the HuCC49 has been replaced with a human CDR sequence.
[c] The values are the percent of complexes, the higher molecular weight species, resolved by size–exclusion chromatography.

Of the six patients analyzed, all six demonstrated reactivity with CDR3 light chain indicating that light chain CDR3 may be immunodominant in murine CC49 MAb. In the heavy chain, CDR2 appears to be dominant but not with the same level of consensus (four of the six patients show the same level of reactivity, the other two

demonstrated partial reactivity). Concordance was obtained among the six patients in regard to CDR2 of the light chain and CDR1 and CDR3 of the heavy chain, which do not appear to contribute to the immunogenicity of the MAb. This is also the case with the light chain CDR1 and, it follows, the variant with the dual substitution of CDR1 and 2 in the light chain, in which all six patients displayed a partial recognition of the variants. Partial recognition with the heavy chain CDR2 variant with two patients may be due to a loss of part but not all of the cognizant epitope, a change in the conformation or conformational epitope, or loss of amino acid residues that might stabilize the antibody:antibody interaction.

Quantitation of Patient Antibody Response

Quantitation of the HAMA or anti–variable region antibody levels in four patients was performed using HPLC analysis. The quantitation study was performed by adding either 500 ng of unlabeled BL–3 or 250 ng of HuCC49, respectively, to the mixture of patient serum and ^{125}I–HuCC49 and calculating the amount of BL–3 or HuCC49 bound in complexes.

As shown in Table 4, below, at 6 weeks, the amount of HAMA varies from patient to patient by 43–fold, while the variability of HAVRA is within 4–fold. Furthermore, the HAMA versus HAVRA levels may vary from 10 to 145–fold. Clearly, HAVRA can be detected at 3 weeks, and, not surprisingly, it does not appear to attain the same levels as HAMA. In patient EA, there is a dramatic 10–fold increase in the level of HAVRA from 6 to 8 weeks that is noteworthy.

Table 4: Quantitation of anti–CC49 variable region and anti–murine response of patients administered ^{177}Lu–CC49

		μg of Ab/ml Sera	
Patient	**Post–Mab Injection**	**BL–3[a]**	**HuCC49[b]**
EA	0	0	0
	3 weeks	4.1	0.3
	6 weeks	289.0	2.3
	8 weeks	314.4	21.6
CP	0	0	0
	3 weeks	16.0	0.8
	5 weeks	25.2	0.7
	6 weeks	23.2	0.7
LQ	0	0	ND
	3 weeks	4.61	0.4
	6 weeks	6.64	0.7
	8 weeks	ND	1.7
JG	0	0	0
	3 weeks	58.6	0.7
	6 weeks	47.8	2.6

Competition Radioimmunoassay

To confirm whether the HAVRA was actually an anti-idiotypic response, including internal image anti-idiotypic antibodies, to the murine MAb CC49, the sera from one patient (EA) was selected and assessed for blocking of the binding of ^{125}I-HuCC49 to BSM in a radioimmunoassay.

The immunoreactivity of the radiolabeled MAbs was assessed using bovine submaxillary mucin (BSM) immobilized on a solid support (Reacti-Gel HW65, Pierce) as a modification of the method reported by Heam et al. (1979), J. Chromatog., 185:463-470 and Schott (1992) Cancer Res., 52:6413-6417. Briefly, bovine submaxillary mucin (BSM), which is TAG-72 positive, was adsorbed to each well of a 96-well polyvinylchloride microtiter plate at 10 ng in 50µl of phosphate buffered saline (pH 7.2) as described by Horan Hand et al. (1992), Cancer Immunol. Immunother., 353:165-174. After treating the wells with 5% BSA in PBS, serial dilutions of the patient sera (25µl in 1% BSA in PBS) were added to each; ^{125}I-CC49 (38 nCi in 25 µl) was also added. Following an 18 hour incubation at 4°C, the plates were washed and the wells counted in a γ-scintillation counter. The percent inhibition was calculated and compared to that of unlabeled CC49. Human IgG (Organon Teknika, Durham, NC), which does not react with TAG-72 was included as a control antibody.

It was found that the patient sera could block the binding of ^{125}I-HuCC49 with BSM (FIG. 22) suggesting that the patient, in actuality, demonstrates an anti-idiotypic response, consisting of the internal image anti-idiotypic antibodies. Furthermore, the anti-idiotypic response was observed to increase over an eight week period. Figure 22 shows the detection of patient (EA) anti-idiotypic antibody response to murine CC49: pre-study sera from patient EA (□); sera collected at 3 weeks (A), 6 weeks (B), and 8 weeks (C).

All references cited in this disclosure are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A humanized anti-TAG-72 antibody comprising:

light chain Complementarity Determining Regions (L-CDRs), comprising L-CDR1, L-CDR2 and L-CDR3; and heavy chain Complementarity Determining Regions (H-CDRs), comprising H-CDR1, H-CDR2 and H-CDR3,

wherein L-CDR3, H-CDR1, H-CDR2 and H-CDR3 are from a non-human antibody and at least one of L-CDR1 and L-CDR2 are human antibody sequences.

2. The humanized antibody of claim 1, wherein L-CDR1 is from a human antibody.

3. The humanized antibody of claim 2, wherein L-CDR1 is from human monoclonal antibody LEN.

4. The humanized antibody of claim 1, wherein L-CDR2 from a human antibody.

5. The humanized antibody of claim 4, wherein L-CDR2 is from human monoclonal antibody LEN.

6. The humanized antibody of claim 1, wherein both L-CDR1 and L-CDR2 are human antibody sequences.

7. The humanized antibody of claim 1, wherein L-CDR1 and L-CDR2 are human antibody sequences from the same human antibody.

8. The humanized antibody of claim 7, wherein L-CDR1 and L-CDR2 are human antibody sequences from human monoclonal antibody LEN.

9. The humanized antibody of claim 6, wherein L-CDR1 and L-CDR2 are human antibody sequences from different human antibodies.

10. The humanized antibody of claim 1, wherein L–CDR3, H–CDR1, H–CDR2 and H–CDR3 are from murine monoclonal antibody CC49.

11. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein at least one amino acid of positions 60, 61, 62, or 64 in H–CDR2 is replaced with a corresponding amino acid from a human antibody.

12. The humanized antibody of claim 11, wherein the human antibody is 21/28'CL.

13. The humanized antibody of claim 11, wherein the amino acid at position 97 of L–CDR3 is replaced with a corresponding amino acid from a human antibody.

14. The humanized antibody of claim 11, wherein at least one of L–CDR1 and L–CDR2 are human antibody sequences.

15. The humanized antibody of claim 14, wherein L–CDR1 is a human antibody sequence.

16. The humanized antibody of claim 15, wherein L–CDR1 is from human monoclonal antibody LEN.

17. The humanized antibody of claim 14, wherein L–CDR2 is a human antibody sequence.

18. The humanized antibody of claim 17, wherein L–CDR2 is from human monoclonal antibody LEN.

19. The humanized antibody of claim 17, wherein both L–CDR1 and L–CDR2 are human antibody sequences.

20. The humanized antibody of claim 19, wherein L–CDR1 and L–CDR2 are human antibody sequences from the same human antibody.

21. The humanized antibody of claim 20, wherein L–CDR1 and L–CDR2 are from human monoclonal antibody LEN.

22. The humanized antibody of claim 19, wherein L–CDR1 and L–CDR2 are human antibody sequences from different human antibodies.

23. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein an amino acid at position 97 of L–CDR3 is replaced with a corresponding amino acid from a human antibody.

24. The humanized antibody of claim 23, wherein at least one amino acid of positions 60, 61, 62, or 64 in H–CDR2 is replaced with a corresponding amino acid from a human antibody.

25. The humanized antibody of claim 23, wherein at least one of L–CDR1 and L–CDR2 are human antibody sequences.

26. The humanized antibody of claim 25, wherein L–CDR1 is a human antibody sequence.

27. The humanized antibody of claim 26, wherein L–CDR1 is from human monoclonal antibody LEN.

28. The humanized antibody of claim 25, wherein L–CDR2 is a human antibody sequence.

29. The humanized antibody of claim 28, wherein L–CDR2 is from human monoclonal antibody LEN.

30. The humanized antibody of claim 25, wherein both L–CDR1 and L–CDR2 are from human antibody sequences.

31. The humanized antibody of claim 30, wherein L–CDR1 and L–CDR2 are human antibody sequences from the same human antibody.

32. The humanized antibody of claim 31, wherein L–CDR1 and L–CDR2 are from human antibody sequences from human monoclonal antibody LEN.

33. The humanized antibody of claim 30, wherein L–CDR1 and L–CDR2 are human antibody sequences from different human antibodies.

34. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein residues at positions 94 and 97 in L–CDR3 are from a non–human anti–TAG–72 antibody.

35. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein residues at positions 31, 32 and 34 in H–CDR1 are from a non–human anti–TAG–72 antibody.

36. A nucleic acid sequence expressing the humanized antibody of any of claims 1, 11, 23, 34 or 35.

37. A vector expressing the humanized antibody of any of claims 1, 11, 23, 34 or 35.

38. A composition for treatment of cancer, comprising the humanized antibody of any of claims 11, 11, 23, 34 or 35.

39. A composition for detecting cancer cells, comprising the humanized antibody of any of claims 1, 11, 23, 34 or 35.

40. A composition of for detecting cancer cells, comprising a polypeptide capable of specifically binding TAG–72, said polypeptide comprising a functional fragment of the humanized antibody of any of claims 1, 11, 23, 34 or 35.

41. The composition of claim 40, wherein the polypeptide comprises a fragment selected from the group consisting of Fv, Fab, and $F(ab')_2$.

42. A method for treating cancer comprising:
administering the humanized antibody of any of claims 1, 11, 23, 34 or 35 to a patient.

43. A method of detecting cancer cells, comprising:
contacting cells with the humanized antibody of any of claims 1, 11, 23, 34 or 35.

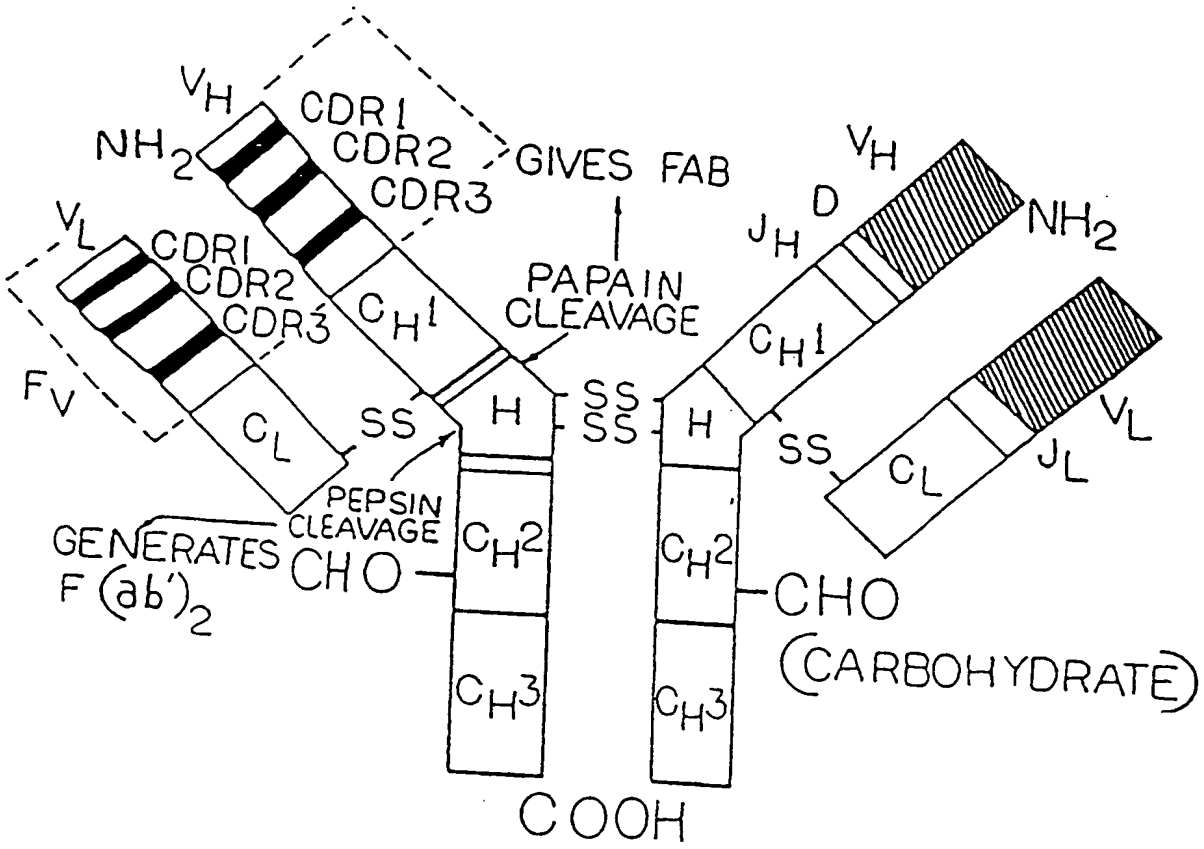
44. The method of claim 43, wherein the humanized antibody is labeled.

45. The method of claim 43, wherein the humanized antibody is detected using a labeled secondary antibody.

46. A method of detecting cancer cells, comprising:

contacting cells with composition comprising a polypeptide capable of specifically binding TAG–72, said polypeptide comprising a functional fragment of the humanized antibody of any of claims 11, 11, 23, 34 or 35.

47. The method of claim 46, wherein the polypeptide comprises a fragment selected from the group consisting of Fv, Fab, and $F(ab')_2$.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J_L
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PEPSIN CLEAVAGE
GENERATES
$F(ab')_2$
CHO
C_H2
C_H2
CHO
(CARBOHYDRATE)
C_H3
C_H3
COOH

Figure 1

Light chain

CDR 1	24	25	26	27	a	b	c	d	e	f	28	29	30	31	32	33	34
HuCC49	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Tyr	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Ala
LEN	Lys	Ser	Ser	Gln	Ser	*Val*	Leu	Tyr	Ser	*Ser*	Asn	*Ser*	Lys	Asn	Tyr	Leu	Ala

CDR 2	50	51	52	53	54	55	56
HuCC49	Trp	Ala	Ser	Ala	Arg	Glu	Ser
LEN	Trp	Ala	Ser	*Thr*	Arg	Glu	Ser

CDR 3	89	90	91	92	93	94	95	96	97
HuCC49	Gln	Gln	Tyr	Tyr	Ser	Tyr	Pro	Leu	Thr
LEN	Gln	Gln	Tyr	Tyr	Ser	*Thr*	Pro	*Tyr*	*Ser*

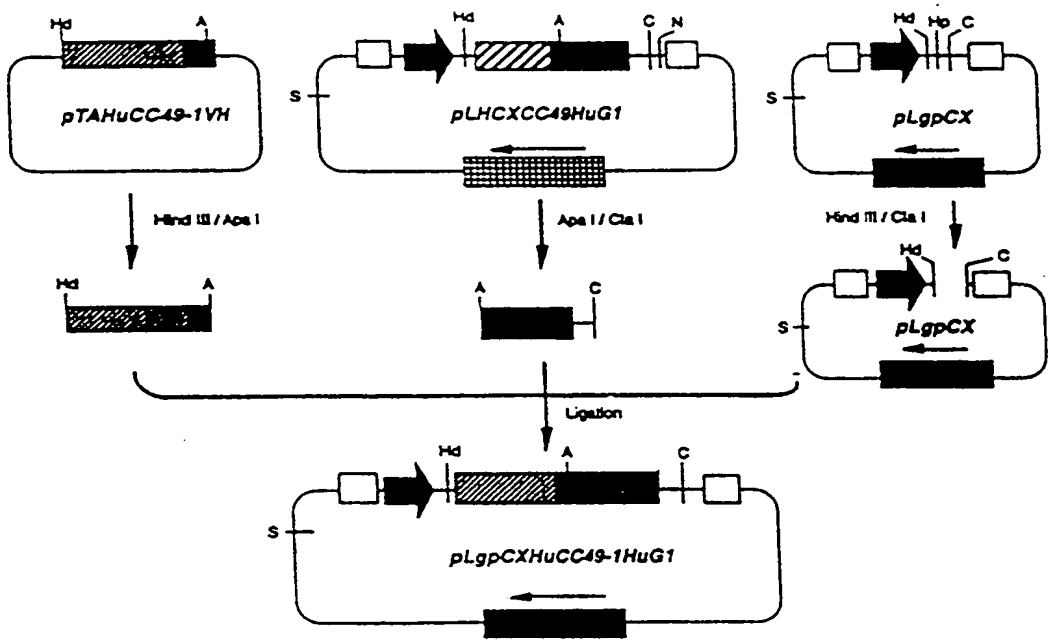
Heavy chain

CDR 1	31	32	33	34	35
HuCC49	Asp	His	Ala	Ile	His
21/28'CL	*Ser*	*Tyr*	Ala	*Met*	His

CDR 2	50	51	52	a	53	54	55	56	57	58	59	60	61	62	63	64	65
HuCC49	Tyr	Phe	Ser	Pro	Gly	Asn	Asp	Asp	Phe	Lys	Tyr	Asn	Glu	Arg	Phe	Lys	Gly
21/28'CL	*Trp*	*Ile*	*Asn*	*Ala*	Gly	Asn	*Gly*	*Asn*	*Thr*	Lys	Tyr	*Ser*	*Gln*	*Lys*	Phe	*Gln*	Gly

CDR 3	95	96	97	98	99	100	a	b	101	102
HuCC49	Ser	Leu	Asn	Met	Ala	-	-	-	-	Tyr
21/28'CL	*Gly*	*Gly*	*Tyr*	*Tyr*	*Gly*	*Ser*	*Gly*	*Ser*	*Asn*	Tyr

Figure 2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C
Hd
Hp
C
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Figure 3

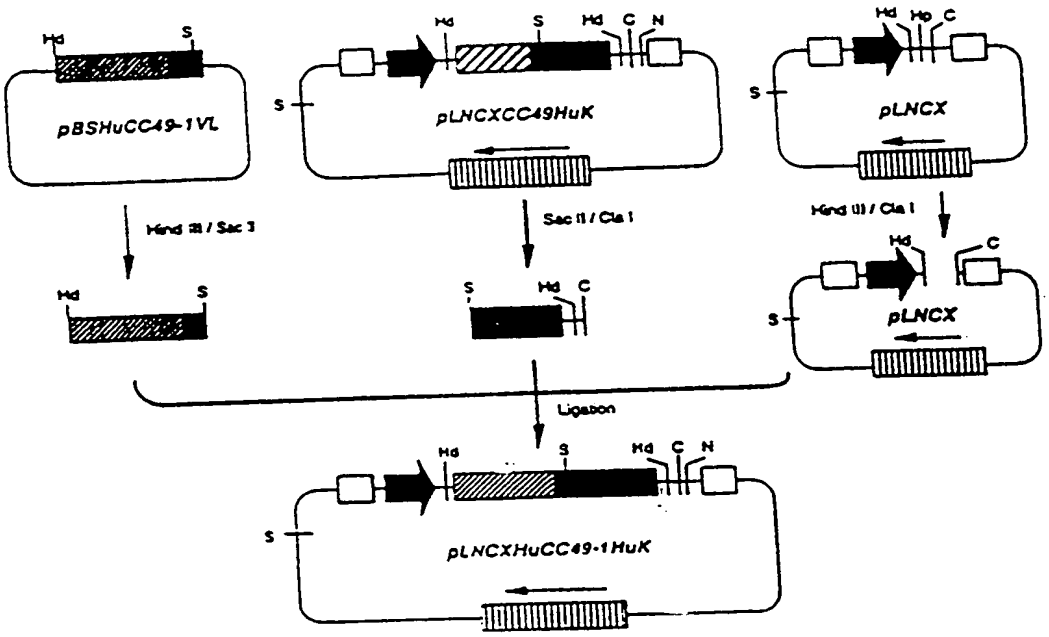
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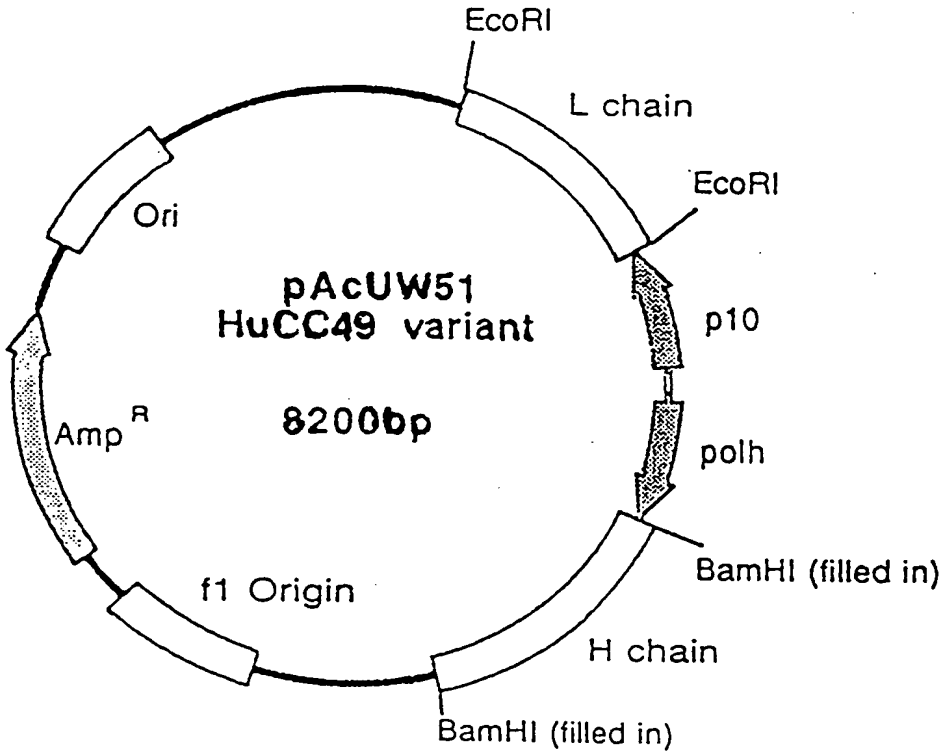
Figure 4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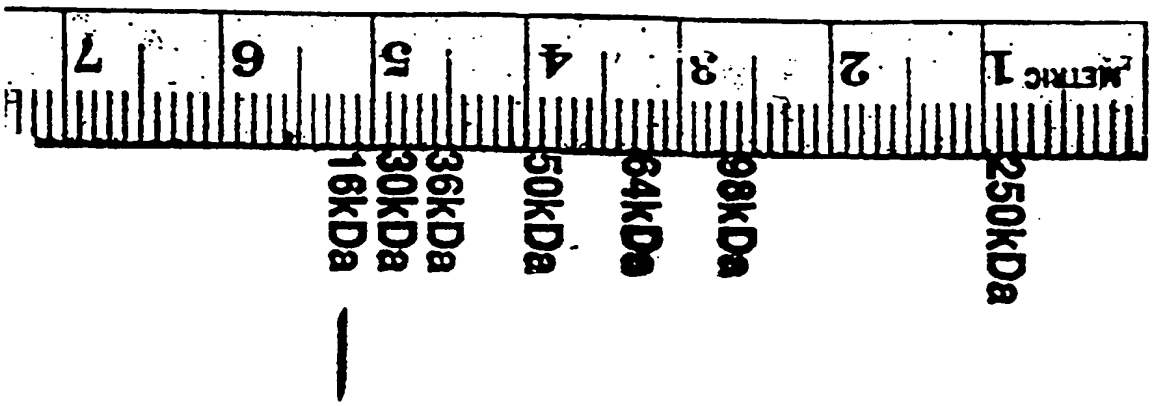
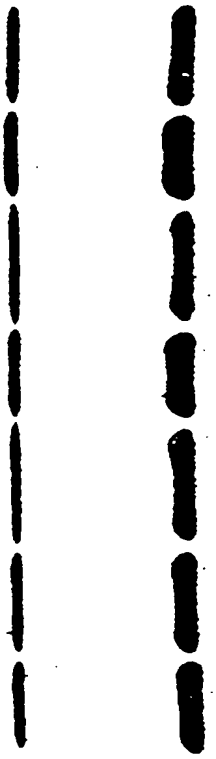
Ori
pAcUW51
HuCC49 variant
8200bp

Figure 5





HuCC49

Lcdr3M97

Lcdr3M94,97

Lcdr3M96

Lcdr3M94

Hcdr1M32,34

Hcdr2M60-62,64

Reduced

Figure 6

Figure 7

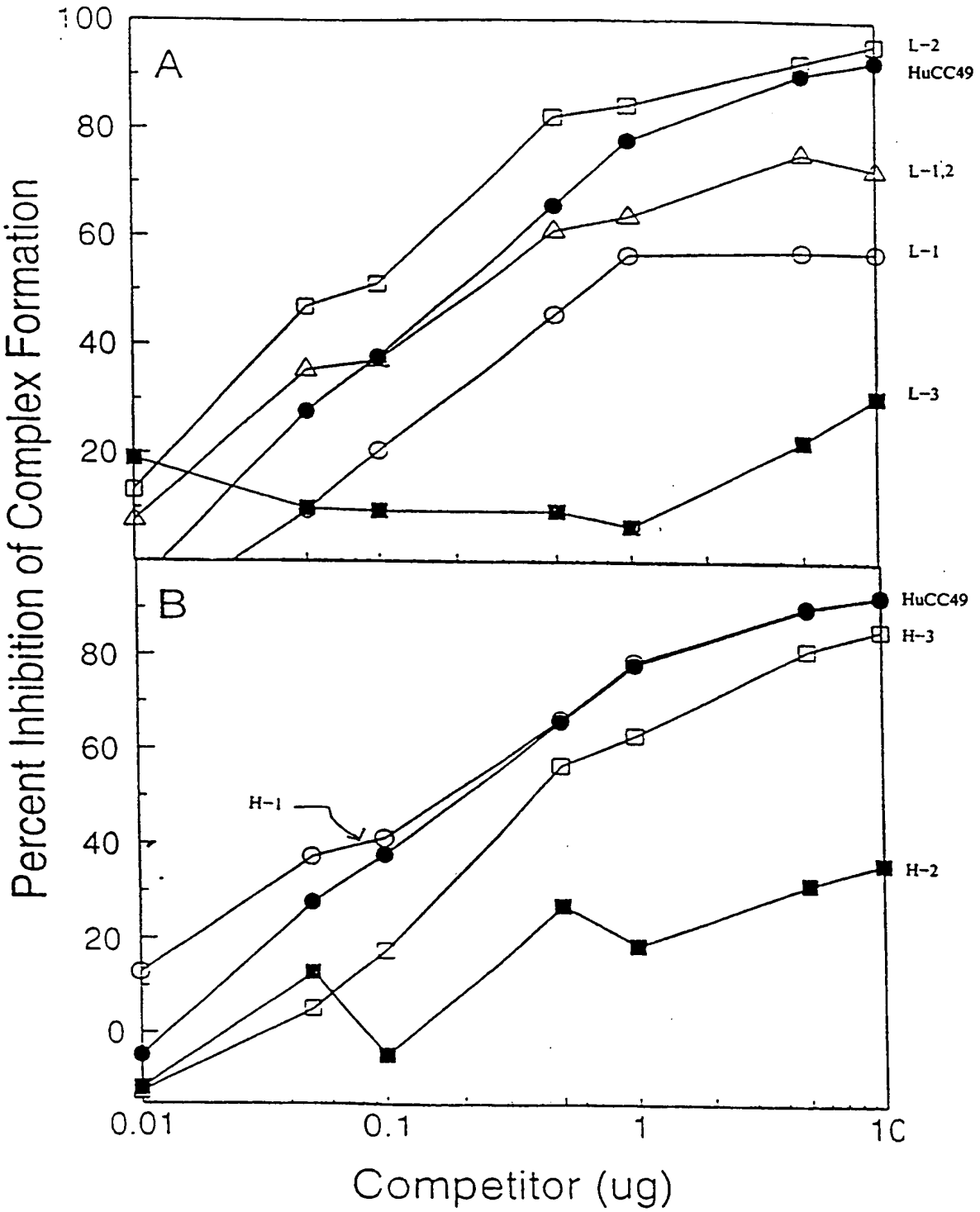

A
B
L-2
HuCC49
L-1,2
L-1
L-3
HuCC49
H-3
H-1
H-2
Percent Inhibition of Complex Formation
Competitor (ug)
0.01
0.1
1
10
0
20
40
60
80
100

Figure 8

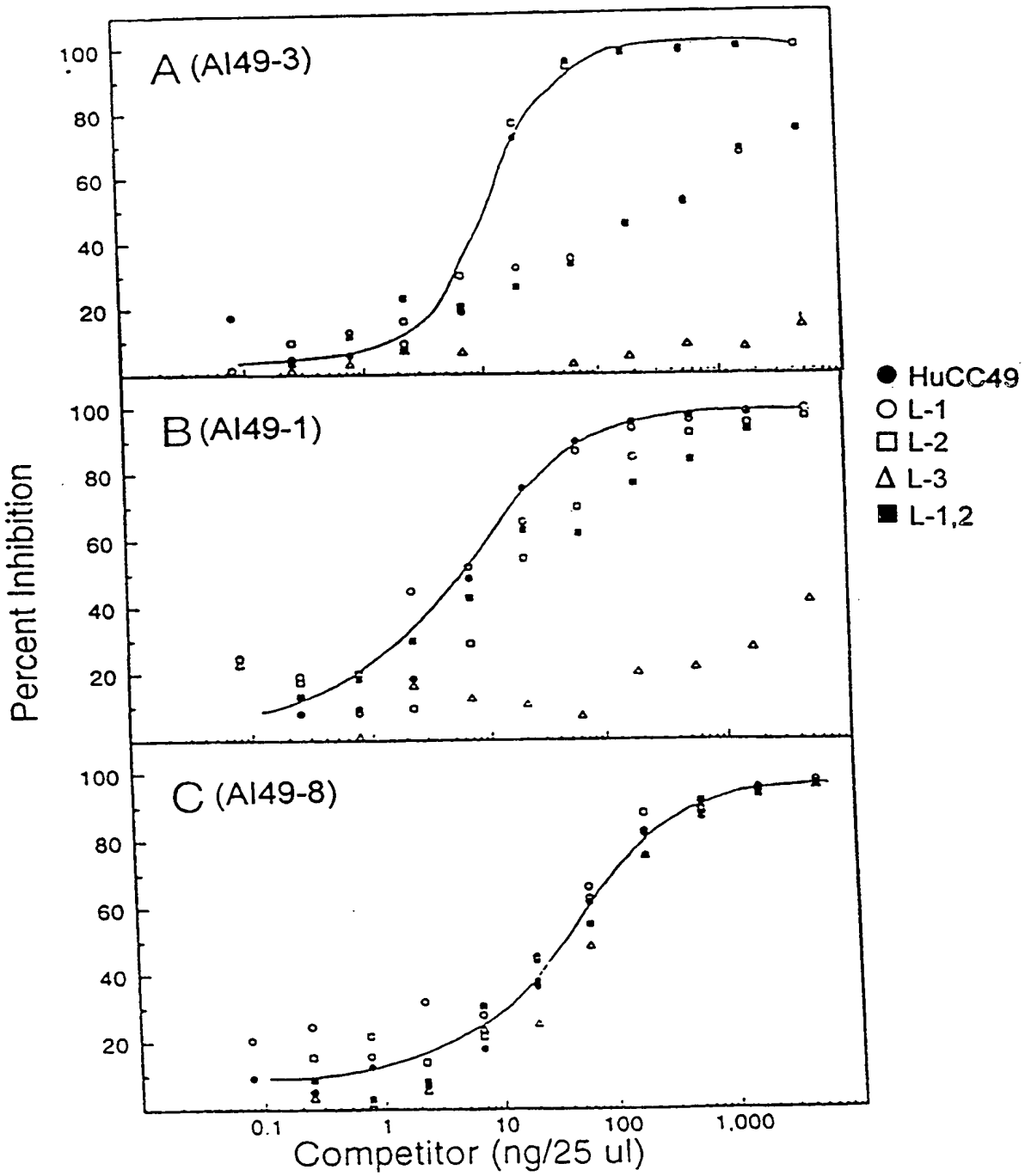

A (AI49-3)
B (AI49-1)
C (AI49-8)
Percent Inhibition
100
80
60
40
20
0.1
1
10
100
1,000
Competitor (ng/25 ul)
HuCC49
L-1
L-2
L-3
L-1,2

Figure 9

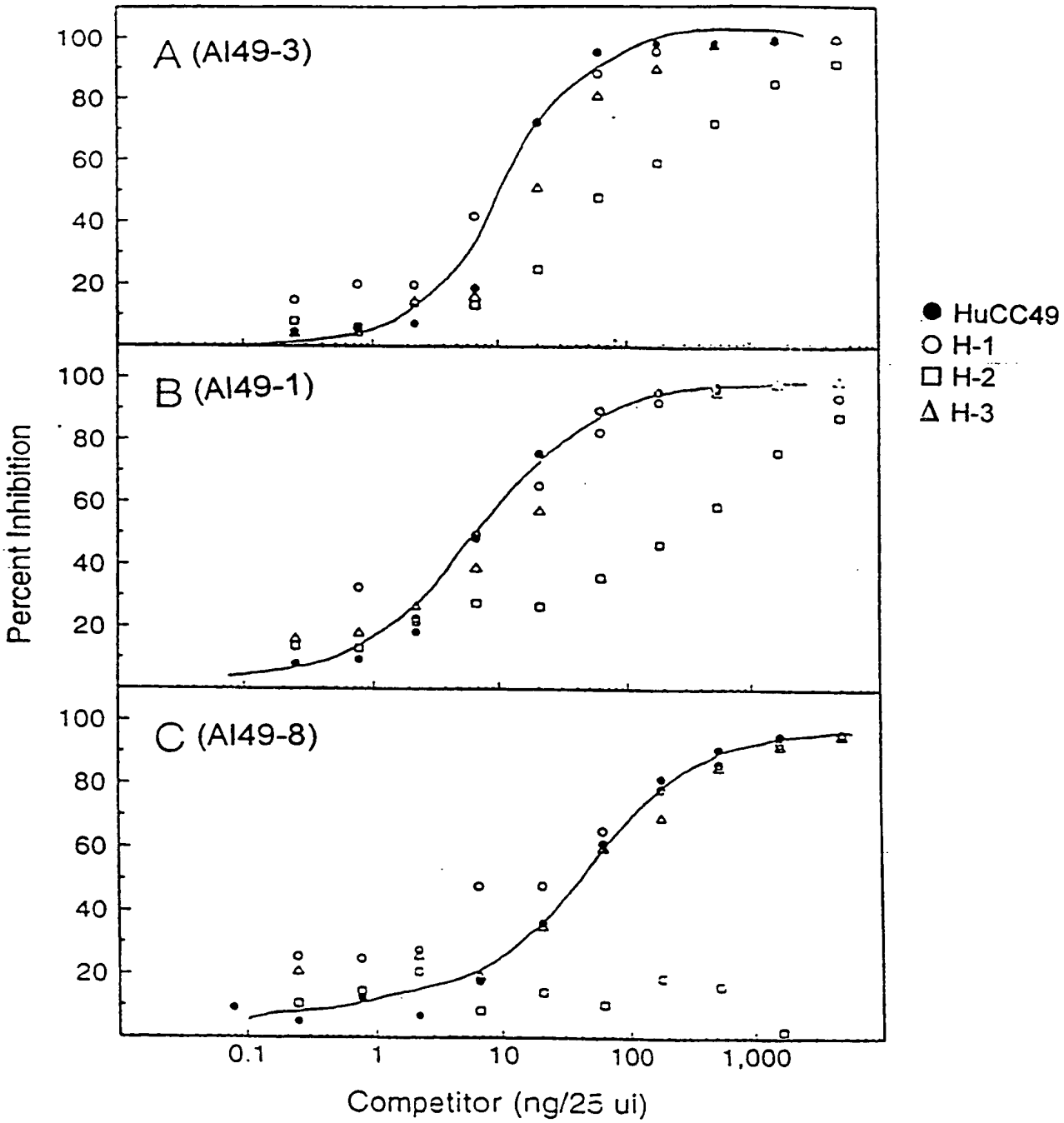
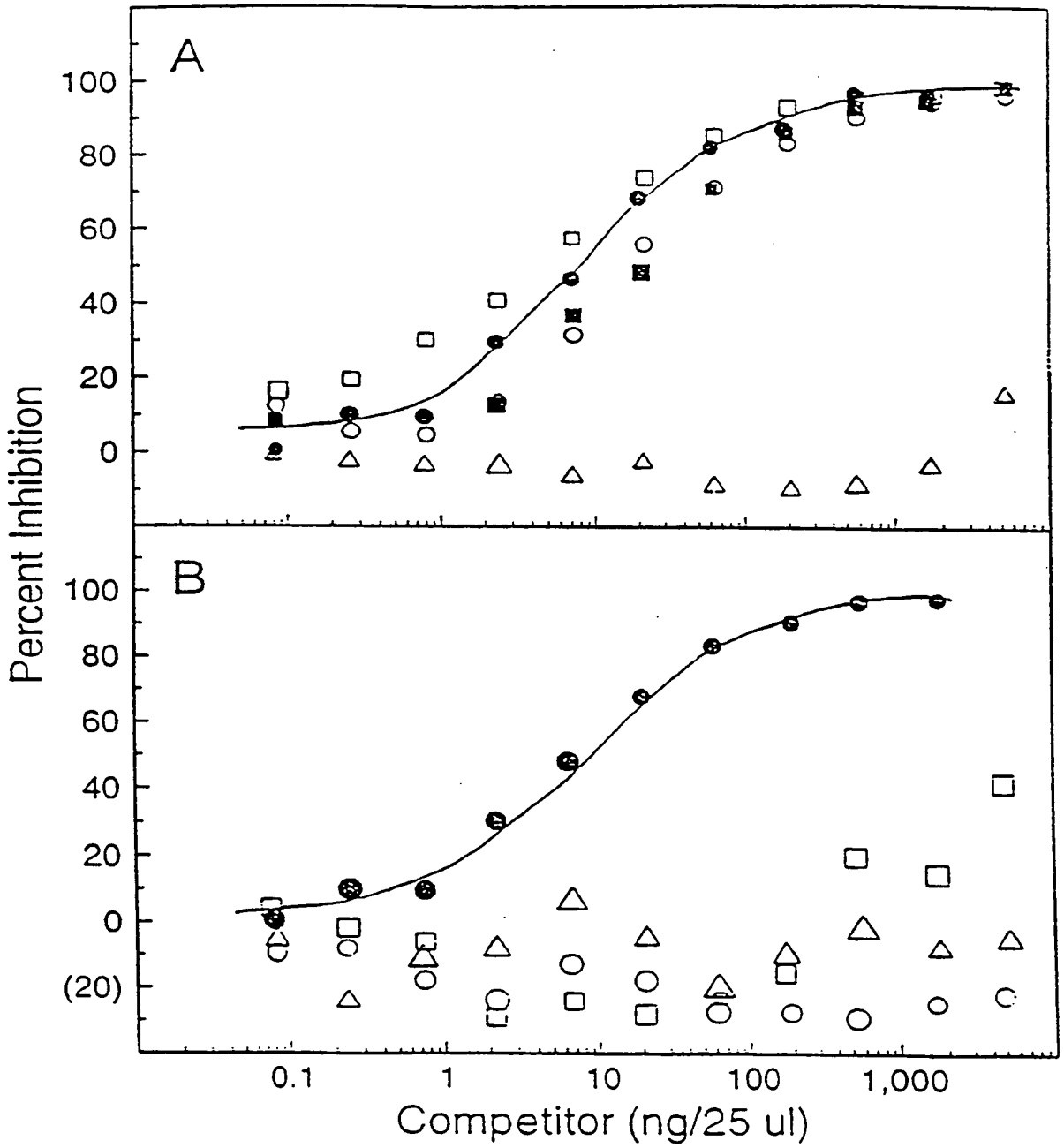

A (AI49-3)
B (AI49-1)
C (AI49-8)
Percent Inhibition
100
80
60
40
20
0.1
1
10
100
1,000
Competitor (ng/25 ul)
HuCC49
H-1
H-2
H-3

Figure 10


A
B
100
80
60
40
20
0
(20)
Percent Inhibition
0.1
1
10
100
1,000
Competitor (ng/25 ul)

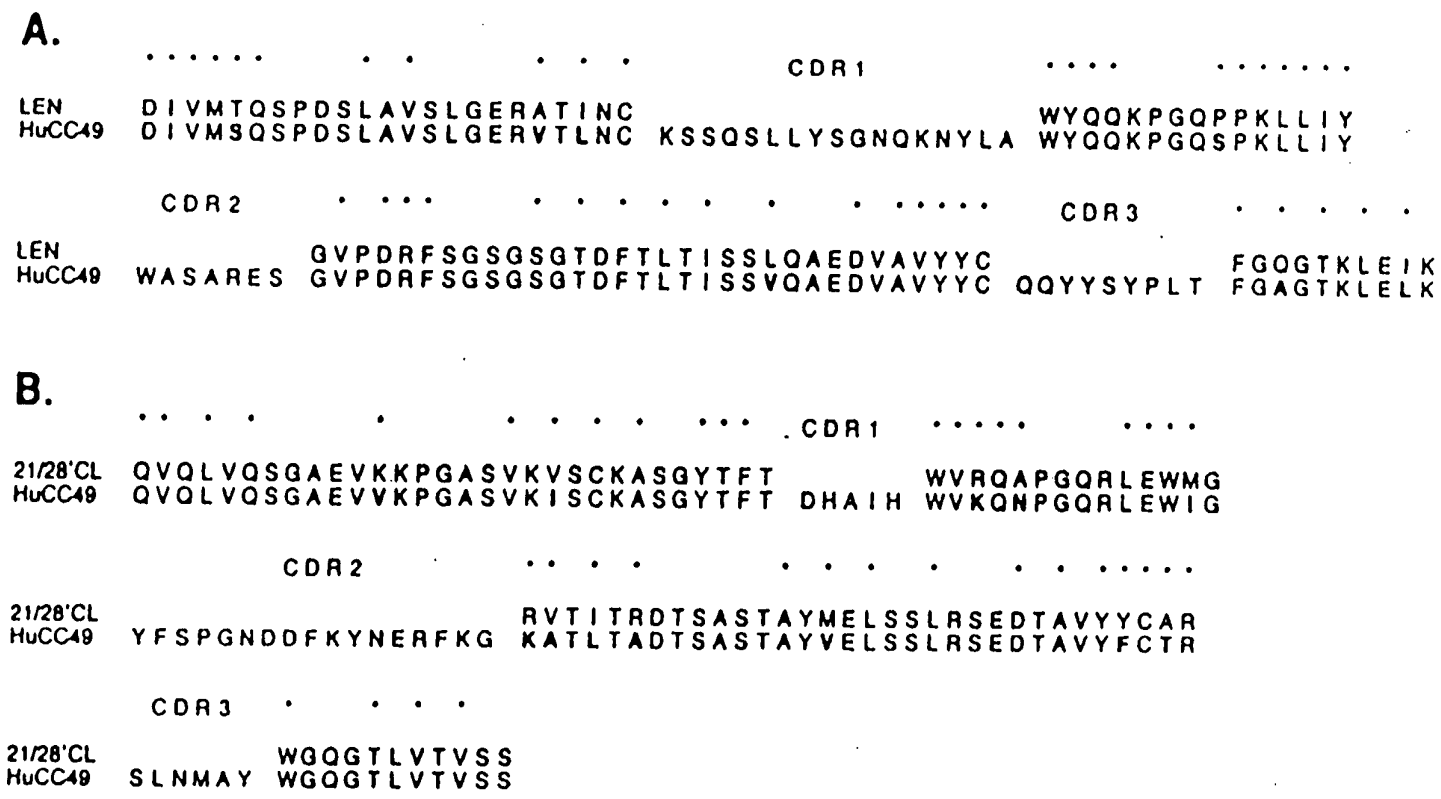
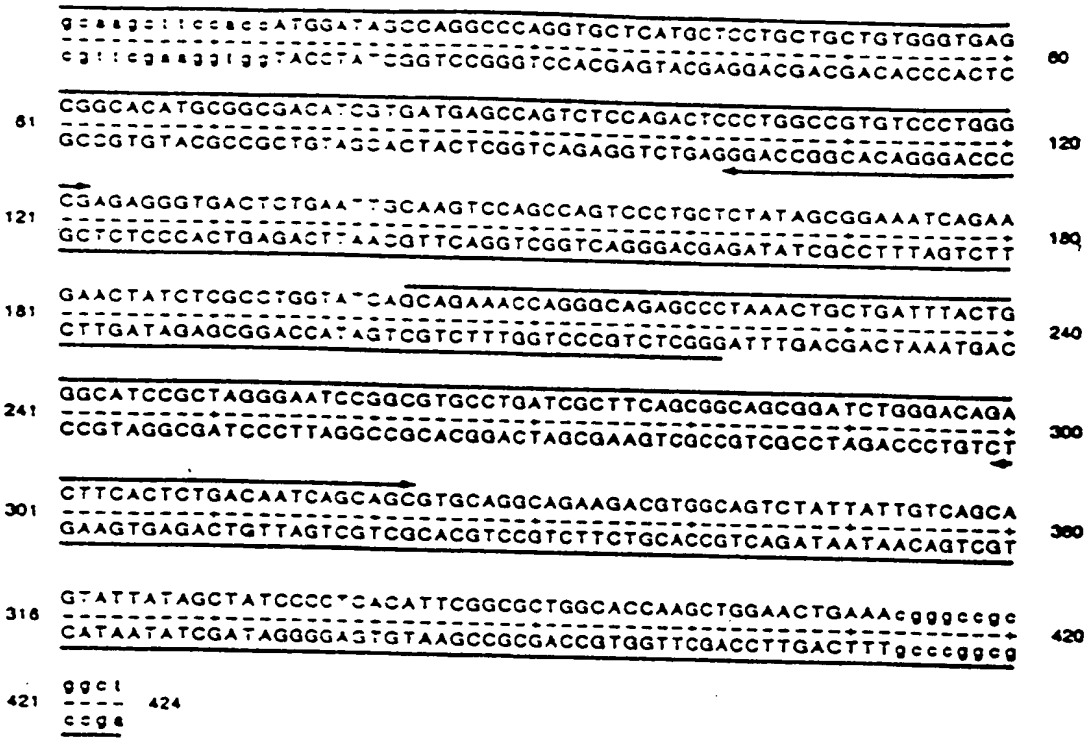

A.
CDR1
LEN DIVMTQSPDSLAVSLGERATINC WYQQKPGQPPKLLIY
HuCC49 DIVMSQSPDSLAVSLGERVTLNC KSSQSLLYSGNQKNYLA WYQQKPGQSPKLLIY
CDR2
CDR3
LEN GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC FGQGTKLEIK
HuCC49 WASARES GVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC QQYYSYPLT FGAGTKLELK
B.
CDR1
21/28'CL QVQLVQSGAEVKKPGASVKVSCKASGYTFT WVRQAPGQRLEWMG
HuCC49 QVQLVQSGAEVVKPGASVKISCKASGYTFT DHAIH WVKQNPGQRLEWIG
CDR2
21/28'CL RVTITRDTSASTAYMELSSLRSEDTAVYYCAR
HuCC49 YFSPGNDDFKYNERFKG KATLTADTSASTAYVELSSLRSEDTAVYFCTR
CDR3
21/28'CL WGQGTLVTVSS
HuCC49 SLNMAY WGQGTLVTVSS

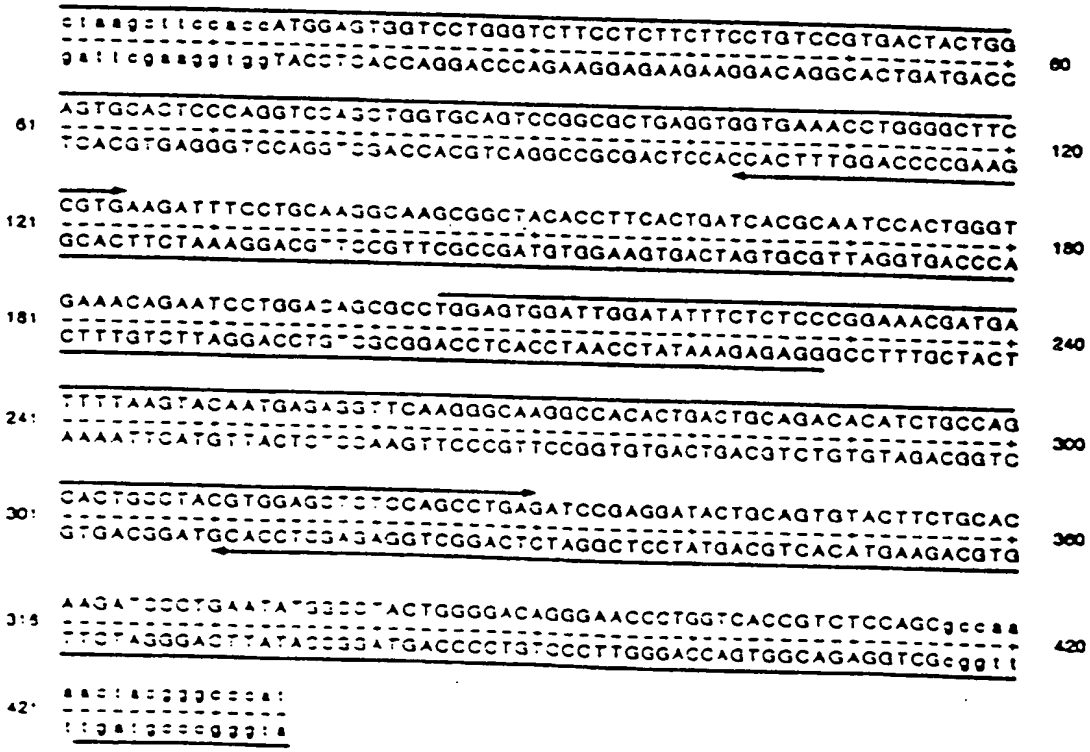
Figure 11

Figure 12

A.



B.



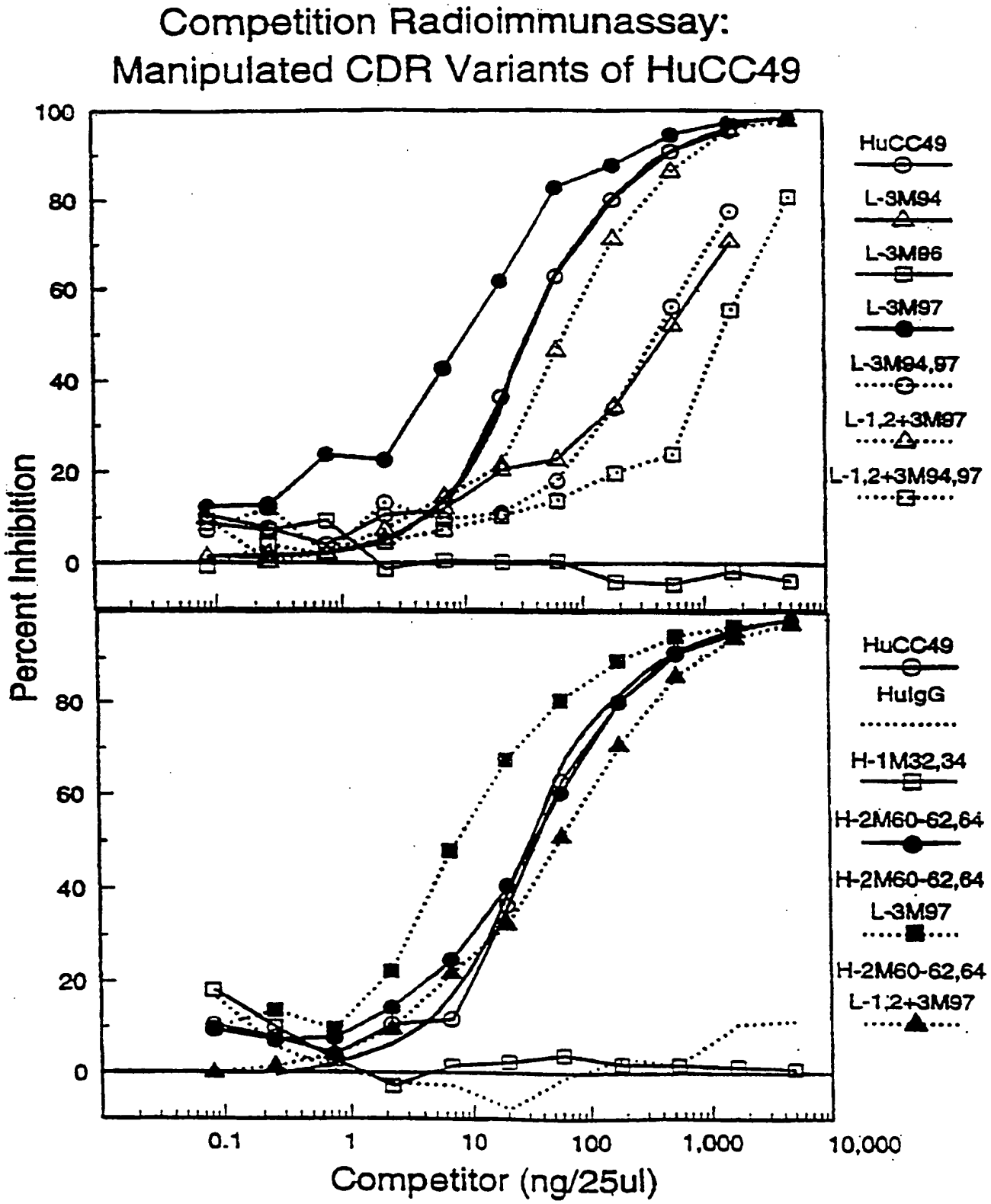

Competition Radioimmunassay:
Manipulated CDR Variants of HuCC49
Percent Inhibition
100
80
60
40
20
0
HuCC49
L-3M94
L-3M96
L-3M97
L-3M94,97
L-1,2+3M97
L-1,2+3M94,97
HuCC49
HuIgG
H-1M32,34
H-2M60-62,64
H-2M60-62,64
L-3M97
H-2M60-62,64
L-1,2+3M97
0.1
1
10
100
1,000
10,000
Competitor (ng/25ul)

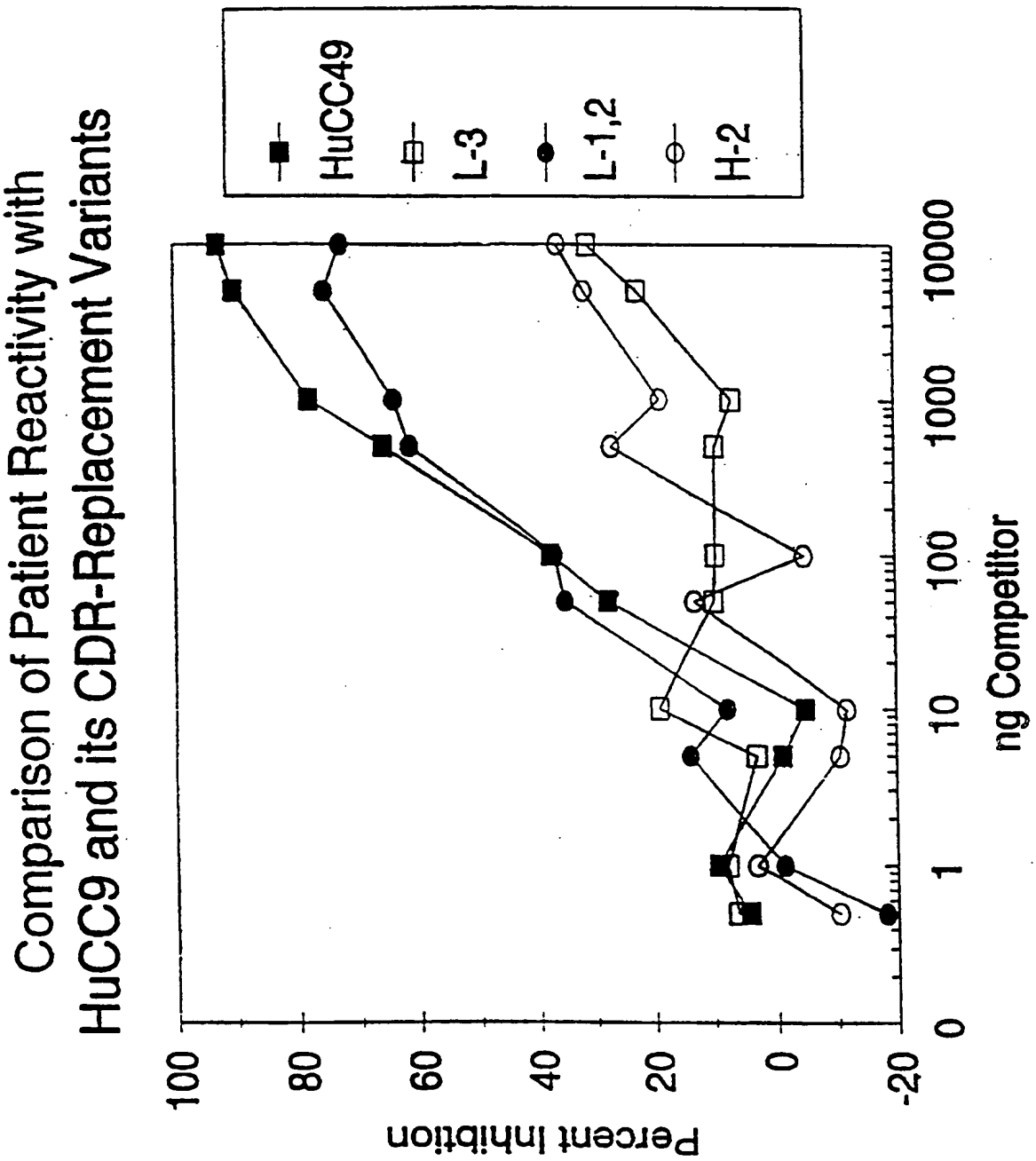
Figure 13

HPLC Analysis of Patient Reactivity to CDR Substitution Variants of HuCC49

Competitor[a]			Patients			
	CDR Substitutions	Antigen Binding	DG	CP	EA	DS
None	—		46.2[b]	32.2	56.8	33.5
HuCC49	—	+++	0	1.5	0.5	0
HuIgG	—	-	59.0	N.D.	63.6	46.4
Light	L3M94	+/-	30.2	20.3	16.4	28.9
	L3M96	-	39.2	31.1	42.9	35.2
	L3M97	+++	0.6	1.3	0.7	2.4
	L3M94,97	+/-	26.5	18.2	18.6	25.6
	L1,2+3M97	++	21.3	17.6	23.8	17.1
	L1,2+3M94,97	+	53.2	38.1	44.2	37.3
Heavy	1M32,34	-	1.4	5.5	3.8	0.7
	2M60-62,64	++	24.4	17.9	21.8	16.5
Both	L3M97 H2M60-62,64	++++	13.0	16.1	3.9	20.1
	L1,2+3M97 H2M60-62,64	++	**33.0**	**30.7**	**24.9**	**32.1**

Figure 14

Figure 15


Comparison of Patient Reactivity with
HuCC9 and its CDR-Replacement Variants
HuCC49
L-3
L-1,2
H-2
Percent Inhibition
100
80
60
40
20
0
-20
0
1
10
100
1000
10000
ng Competitor

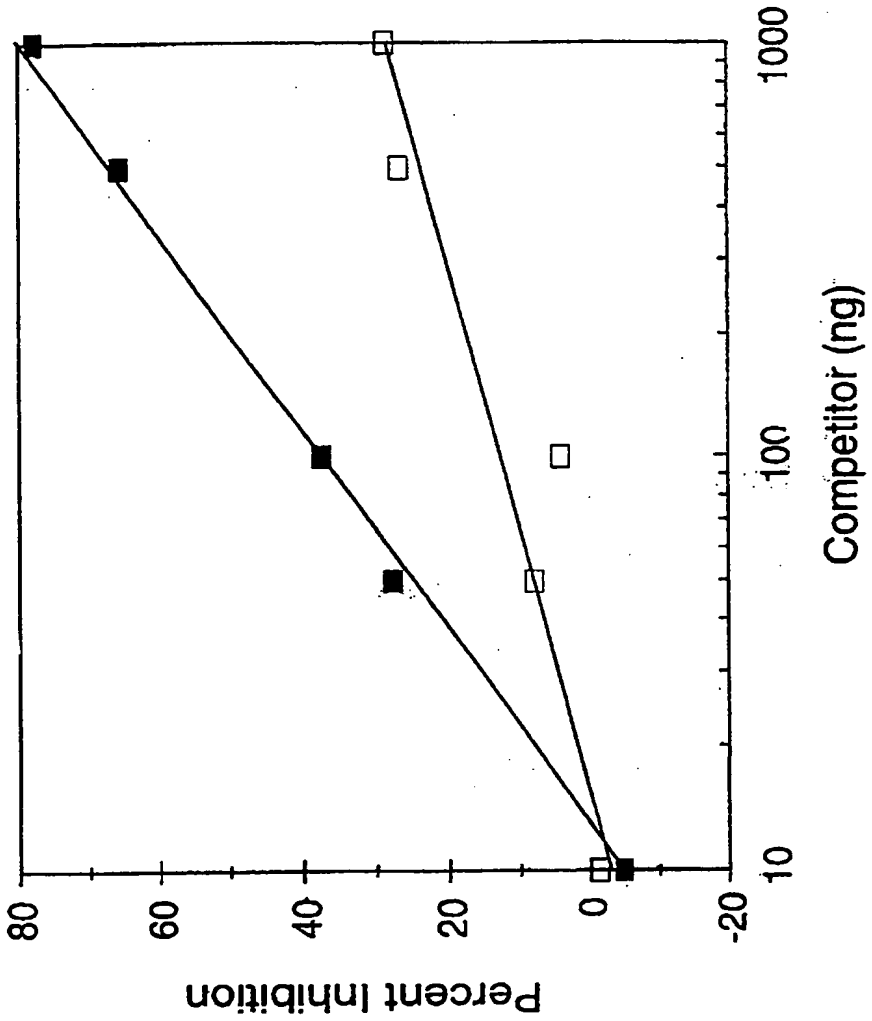


Fig 1a

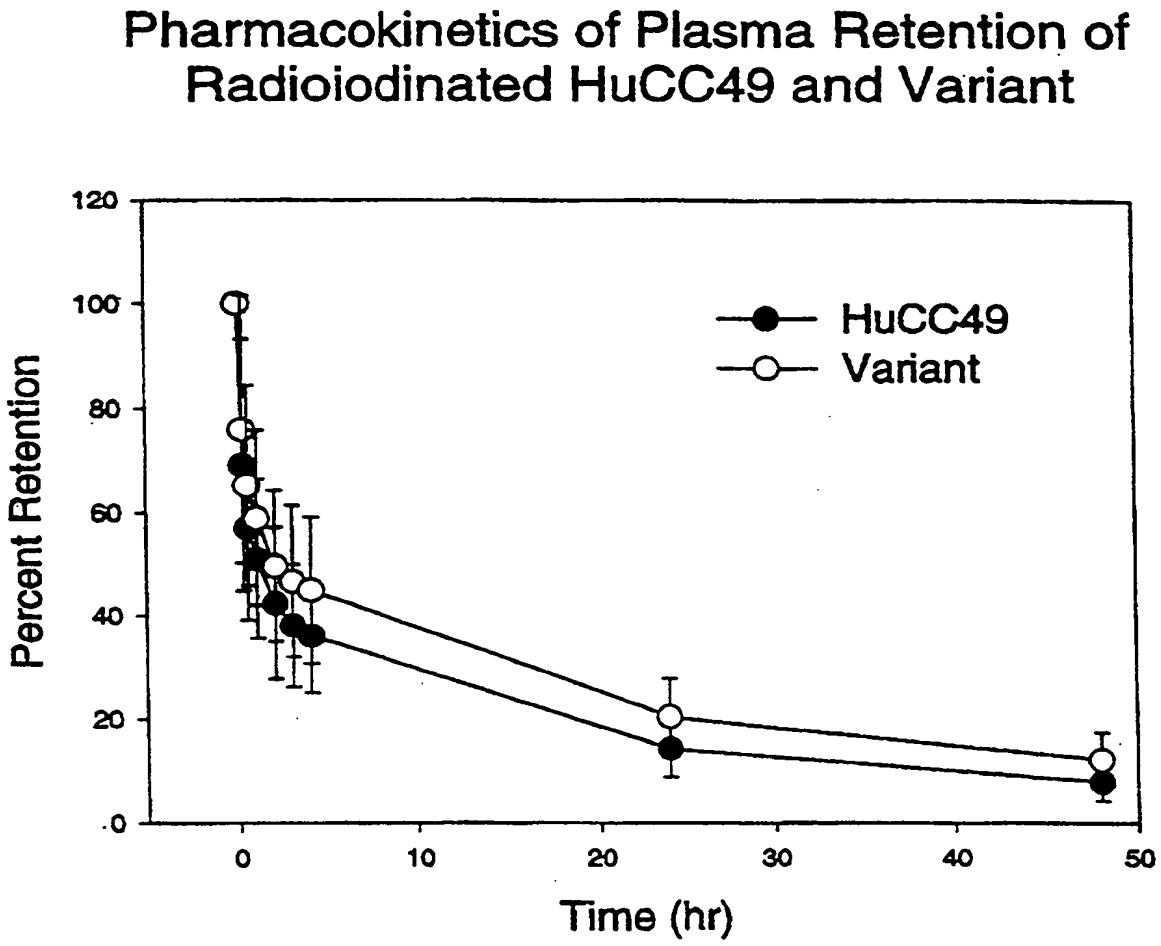

Pharmacokinetics of Plasma Retention of Radioiodinated HuCC49 and Variant
Percent Retention
120
100
80
60
40
20
0
0
10
20
30
40
50
Time (hr)
HuCC49
Variant

Figure 17

Biodistribution of i.v. administered radiolabeled HuCC49 and Variant in athymic mice bearing LS-174T human colon carcinoma xenografts: Percent of injected dose/gram[a]

		Timepoints (hr)				
Antibody	Organ	24	48	72	120	168
Variant	Tumor	15.83	23.75	21.01	17.74	9.21
	Blood	6.35	4.93	4.88	2.19	0.63
	Liver	3.39	2.14	1.46	0.91	0.32
	Spleen	5.90	6.04	2.55	2.43	3.96
	Kidney	2.52	1.27	1.00	0.77	0.36
	Lung	3.22	2.57	2.50	1.12	0.36
HuCC49	Tumor	11.86	17.59	15.31	13.75	5.24
	Blood	4.17	2.94	2.85	1.29	0.18
	Liver	4.77	3.05	1.41	0.70	0.12
	Spleen	6.41	7.47	2.28	2.00	0.46
	Kidney	1.86	0.92	0.70	0.57	0.14
	Lung	2.17	1.58	1.46	0.68	0.12

Figure 18

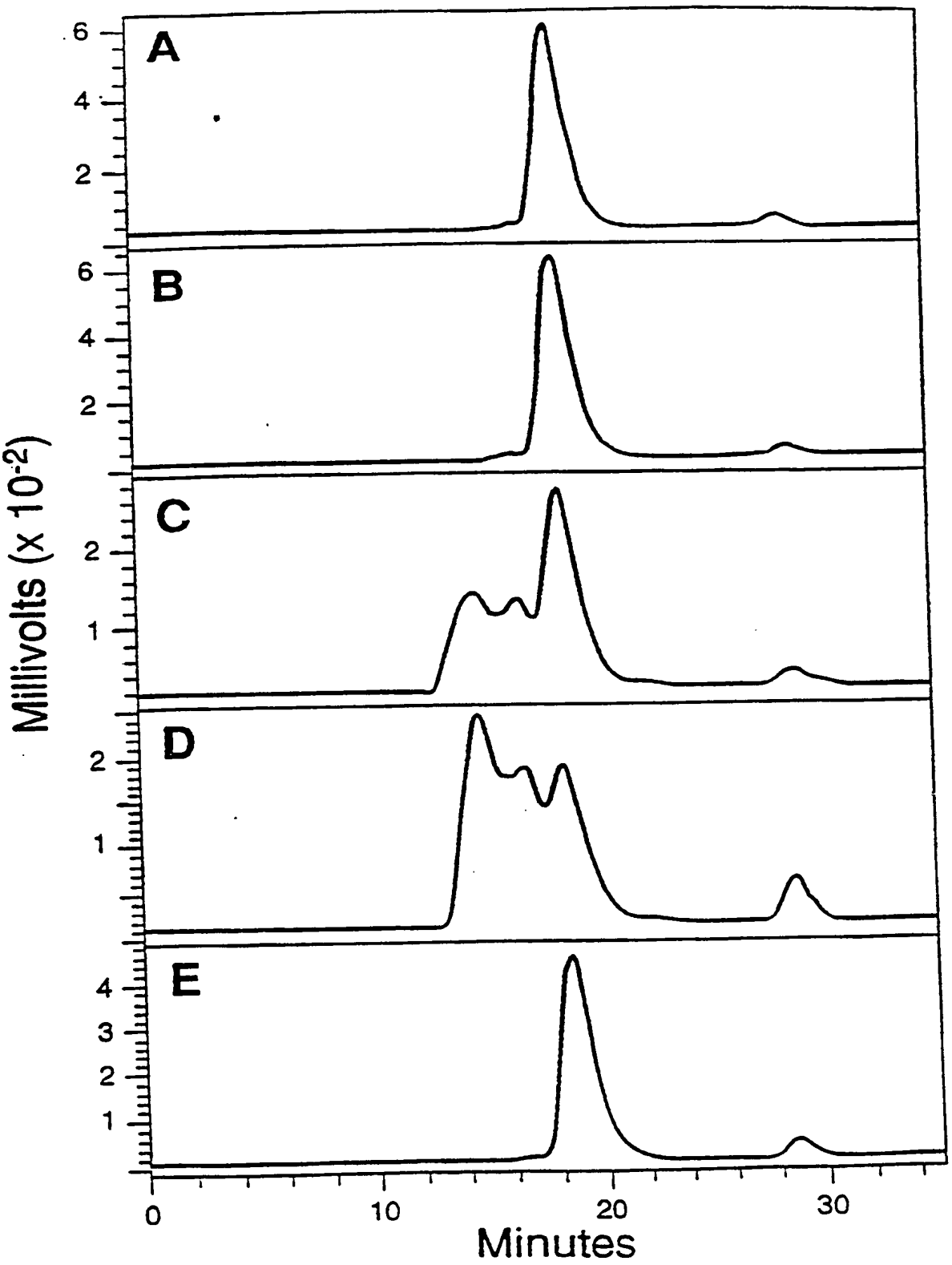


Fig 19

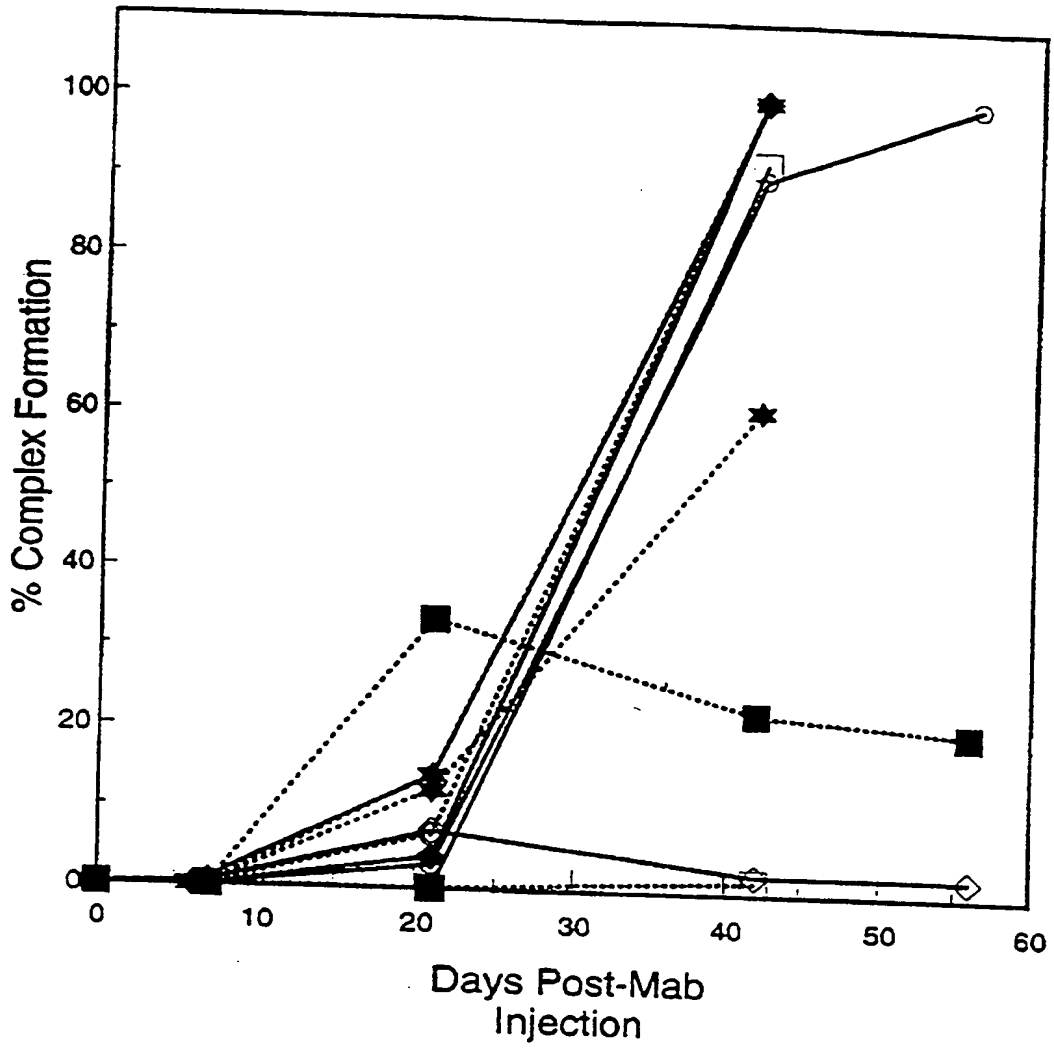

% Complex Formation
100
80
60
40
20
0
0
10
20
30
40
50
60
Days Post-Mab Injection

Fig 20

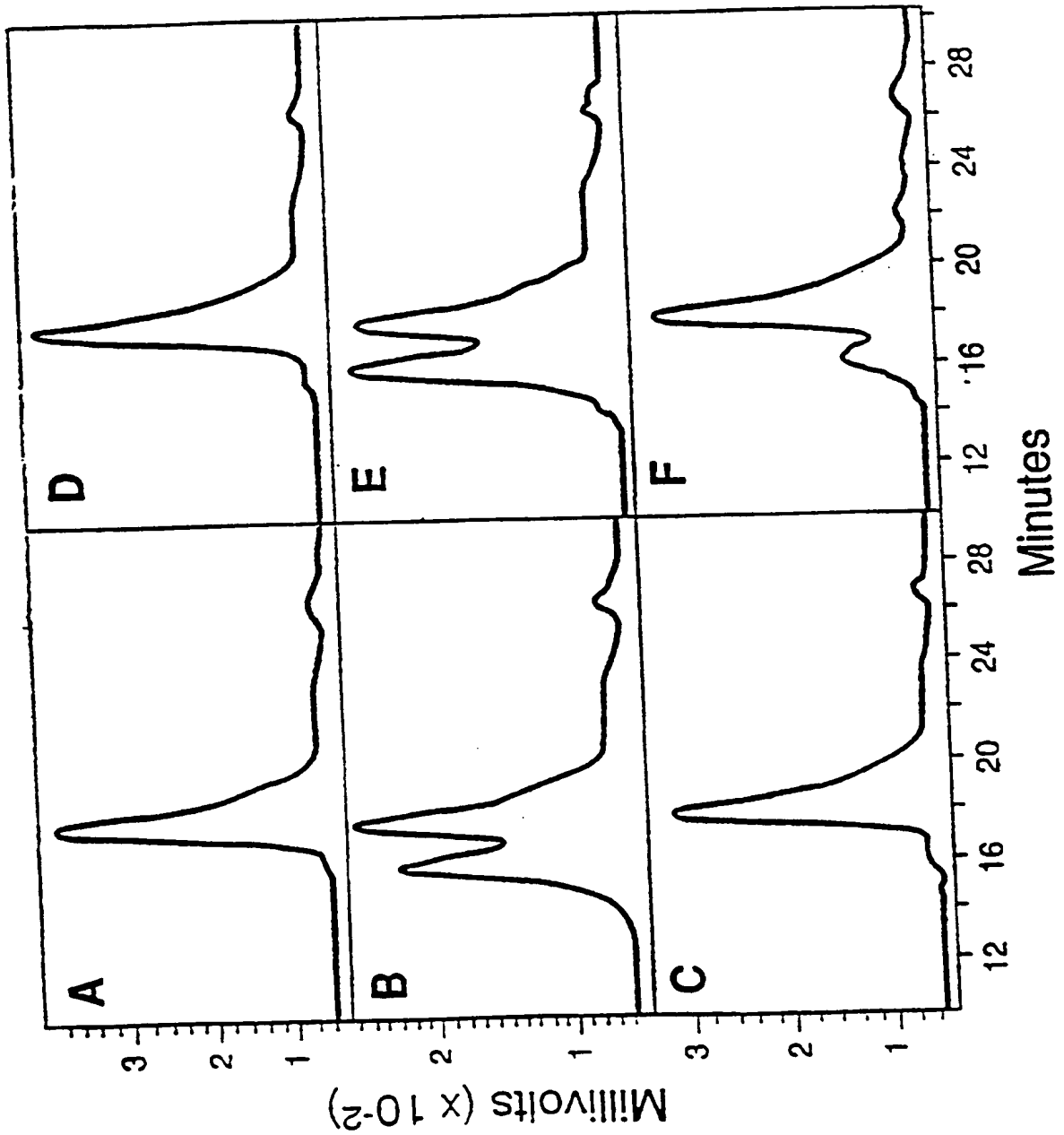


Fig 21

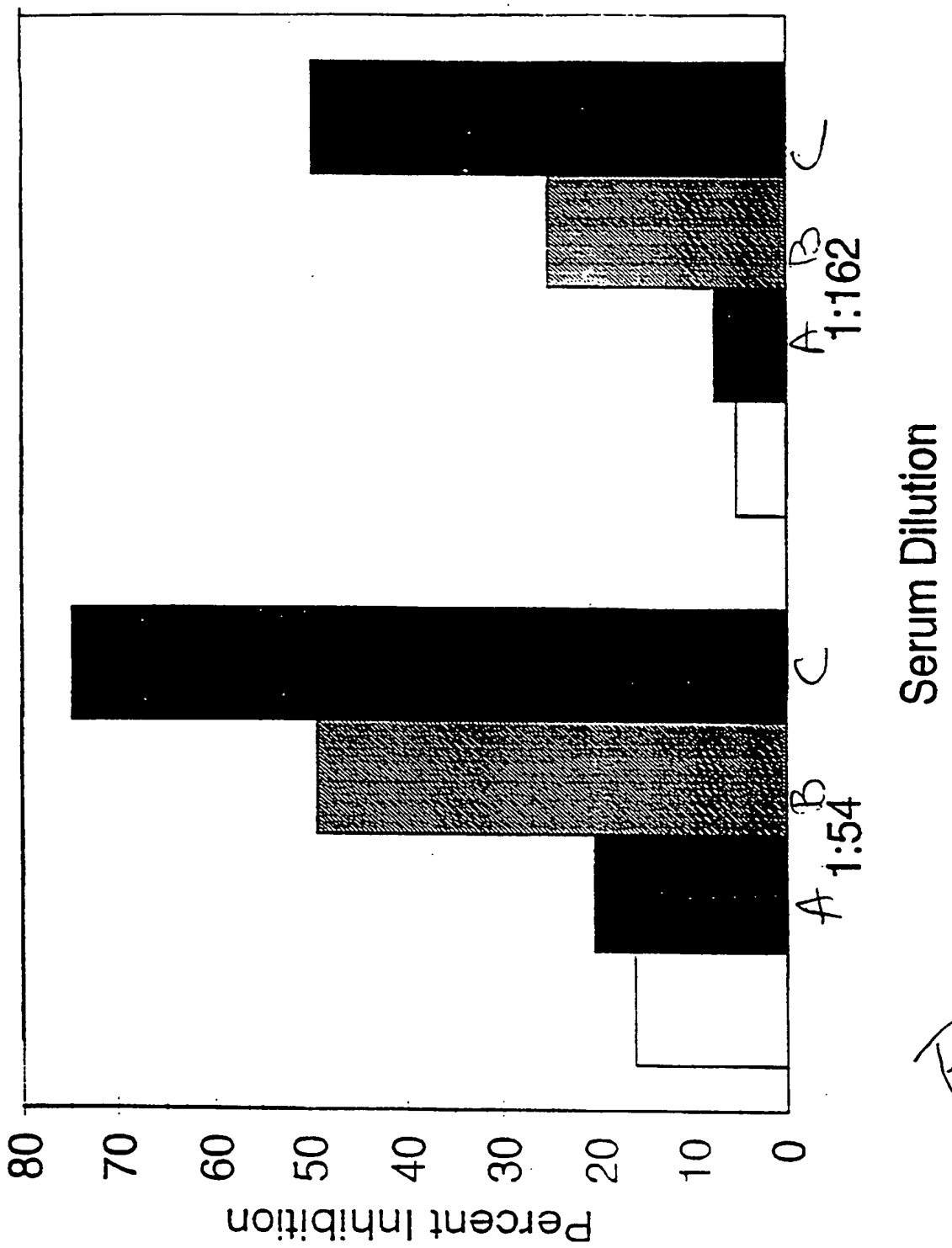

Percent Inhibition
0
10
20
30
40
50
60
70
80
A
B
C
1:54
A
B
C
1:162
Serum Dilution

FIG 2J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/25552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N15/62 C12N5/10 C07K16/30 C07K16/46
A61K51/10 A61P35/00 G01N33/574 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y SHA ET AL: "A heavy-chain grafted antibody that recognizes the tumor-associated TAG72 antigen" CANCER BIOTHERAPY, vol. 9, no. 4, 1 January 1994 (1994-01-01), pages 341-349, XP002079337 abstract page 342, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 346, left-hand column, paragraph 2 -page 347, right-hand column, paragraph 1 ---	1-47
X	WO 97 26010 A (SMITHKLINE BEECHAM CORP., USA;UNIVERSITY OF VERMONT AND STATE AGRICULT) 24 July 1997 (1997-07-24) page 9, line 28 -page 10, line 10 page 21, line 25 -page 22, line 13 --- -/--	1,2,4,6, 7,9, 36-41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: "Complementarity determining region residues aspartic acid at H55, serine at H95 and tyrosines at H97 and L96 play important roles in the B72.3 antibody-TAG72 antigen interaction." retrieved from STN Database accession no. 97015918 XP002134981 abstract & PROTEIN ENGINEERING, (1996 JUN) 9 (6) 539-43. , ---	23,36-47
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: "The tyrosine residue at position 97 in the VH CDR3 region of a mouse/human chimeric anti-colorectal carcinoma antibody contributes hydrogen bonding to the TAG72 antigen." retrieved from STN Database accession no. 95102752 XP002134982 abstract & CANCER BIOTHERAPY, (1993 FALL) 8 (3) 253-62. , ---	23,36-47
A	WO 96 13594 A (US HEALTH) 9 May 1996 (1996-05-09) page 24, line 9 -page 26, line 3 examples 13,17,18 ---	1-47
P,A	WO 99 43816 A (ARMOUR KATHRYN ;CARR FRANK J (GB); HARRIS WILLIAM J (GB); TEMPEST) 2 September 1999 (1999-09-02) example 1 claims ---	1-47
T	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US IWAHASHI M ET AL: "CDR substitutions of a humanized monoclonal antibody (CC49): contributions of individual CDRs to antigen binding and immunogenicity." retrieved from STN Database accession no. 2000162136 XP002134983 abstract & MOLECULAR IMMUNOLOGY, (1999 OCT-NOV) 36 (15-16) 1079-91. , --- -/--	1-47

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	TAMURA M ET AL: "Structural correlates of an anticarcinoma antibody: identification of specificity-determining residues (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only." JOURNAL OF IMMUNOLOGY, (2000 FEB 1) 164 (3) 1432-41. , XP000901556 the whole document	1-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 25552

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 42 is directed to a method of treatment of the human/animal body and claims 43-47 (all partially) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/25552

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726010 A	24-07-1997	AU 706397 B	17-06-1999
		AU 1830897 A	11-08-1997
		CN 1213312 A	07-04-1999
		HU 9900396 A	28-05-1999
		NO 983284 A	16-09-1998
		PL 327929 A	04-01-1999
		US 6005091 A	21-12-1999
		ZA 9700347 A	06-10-1998
WO 9613594 A	09-05-1996	US 5889157 A	30-03-1999
		US 5981726 A	09-11-1999
		US 5608039 A	04-03-1997
		AU 4135596 A	23-05-1996
		CA 2203236 A	09-05-1996
		EP 0796334 A	24-09-1997
		JP 10508202 T	18-08-1998
		US 5990296 A	23-11-1999
WO 9943816 A	02-09-1999	AU 6439398 A	15-09-1999

(Colcher et al. (1990), J. Nucl. Med., 31:1133–1142) served as a positive control. The patients' sera were demonstrated to have antibodies against the variable region of the murine CC49.

Figure 19 shows an HPLC analysis of patient HAMA following intravenous injection of ^{177}Lu–CC49. Serum samples from LQ were analyzed for the presence of HAMA at various timepoints before and after injection with 20 mg of ^{177}Lu–labeled CC49. Pre–study sera (A), sera collected at 7 days (B), 3 weeks (C), and 6 weeks (D) were mixed with ^{125}I–BL–3 and applied to a size exclusion column. Reduction in retention time of the radiolabeled BL–3 as compared to migration of the ^{125}I–BL–3 in buffer (E) were indicative of immune complex formation and therefore the presence of HAMA.

Lack of complex formation is evident (FIG. 19A) when the pre–study sera of Patient LQ is incubated with the ^{125}I–BL–3. All of the radioactivity is associated with the peak at about 18.5 minutes, the same retention time for ^{125}I–BL–3 in buffer (FIG. 19E). Complex formation is also absent when the sera collected at seven days is incubated with ^{125}I–BL–3 (FIG. 19B). With sera collected at 3 weeks (FIG. 19C), however, there is an indication of complex formation (46%) with the appearance of two peaks with a shorter retention time (i.e., 14 and 16 minutes). The peaks at a shorter retention time indicate the development of a higher molecular weight species in the sera. At 6 weeks (FIG. 19D), the HAMA response has increased, the amount of radioactivity bound in complexes is now 66%.

Figure 20 shows an HPLC analysis of patients' humoral response to the variable region of MAb CC49. The percent complex formation has been plotted versus time for (solid lines) patients DS (O), LW (□), JJ (Δ), DG (●), LJ (■), TD(▲); (dotted lines) JG (O), RW (□), JM (Δ), EA (●), CP (■), LQ (▲);

At one week, none of the patients showed a detectable response against the HuCC49 (FIG. 20). At 3 weeks, sera from nine of the twelve patients (75%) appears to contain antibody against the variable region of CC49 with one patient having a notably higher response than the others. For the eleven patients evaluated at six weeks, only two patients did not elicit a human antivariable region antibody response (HAVRA) against CC49, i.e., 9 of 11 evaluable patients (82%) had antibody against the variable region of the murine MAb CC49.

Three patterns of HAMA–HAVRA response are evident. The patterns of the HAMA and HAVRA responses elicited in each of the patients were very similar, differing only in the apparent level of antibody. Patients DG, LW, LQ and CP

developed HAVRA simultaneously with HAMA. Patients DS and JM appear to have a strong HAVRA, while HAMA response is modest. While in patients TD, JG, and EA, the HAVRA level is lower than HAMA at 3 weeks, followed by HAMA and HAVRA attaining high levels at later timepoints. In no patient was there a HAVRA response without the development of HAMA..

The HAMA results for the twelve patients are summarized below in Table 2.

Table 2: HPLC Analysis of Patients' Anti-mouse immunoglobulin response after i.v. injection of ^{177}Lu-CC49

	Days Post-Injection of ^{177}Lu-CC49			
Patient	**7**	**21**	**42**	**56**
DS	0[a]	1	16	27
LW	3	6	81	NA
JJ	0	12	3	4
DG	0	24	84	NA
LJ	0	42	NA	NA
JM	0	8	47	NA
JG	4	83	83	NA
RW	0	1	2	NA
TD	0	95	100	NA
EA	0	27	100	100
CP3	0	33	27	NA
LQ	0	46	66	100

[a] The values are the percent of ^{125}I-BL-3 detected in complexes after a brief incubation with the patient sera and resolved by size-exclusion chromatography. The timepoints of each patient are background corrected using the patients' pre-study sera.

The patterns of the HAMA responses are varied and are consistent with previous findings by Colcher et al. (1990), J. Nucl. Med. 31:1133–1142. Ten out of the twelve patients (83%) demonstrate a HAMA response at 3 weeks following a single intravenous injection of 20 mg ^{177}Lu–labeled CC49, two patients (LW and JG) have minimal responses evident at 7 days with complexes of 3% and 4%, respectively. One patient (RW) may be considered a nonresponder. Some of the patients show an escalating HAMA response, while others plateau. Yet another (JJ) peaks at 3 weeks, followed by an apparent decrease in the HAMA level. Overall, at 3 weeks, 8 of 12 patients (57%) at and 6 weeks, 9 of 11 (82%) were HAMA positive.

Specificity of Patient Response

The specificity of the patients' antibody response to CC49 was assessed using ^{125}I–labeled HuCC49 and HuCC49 CDR–replacement variants to determine whether or not any of the responses were directed against the variable region of CC49. To accomplish this, the HPLC methodology was employed using ^{125}I–HuCC49 as the probe (See, Kashmiri et al. (1995), Hybridoma, 14:461–473).

To eliminate the artifactual influence of TAG–72 in the HPLC analysis for anti–CC49 antibody responses found in the patient's serum, immunoadsorbents were prepared as reported by Ferroni et al. (1992) J. Clin. Lab. Analysis, 4:465–473. For the purpose of these studies, purified MAb CC92 was coupled to Reacti–gel (HW65F, Pierce) according to the method of Heam et al. (1979), J. Chromatog., 185:463–470. MAb CC92 is a second–generation monoclonal antibody that reacts with TAG–72, but with an epitope distinct from the one recognized by CC49.

Before probing the patients' sera with the ^{125}I–HuCC49, removal of HAMA and circulating TAG–72 were confirmed using ^{125}I–BL–3 and ^{125}I–B72.3, respectively (data not shown). MAb B72.3 is an anti–TAG–72 MAb that has been shown to form complexes with TAG–72 in patient sera (Colcher et al. (1990), J. Nucl. Med., 31:1133–1142).

In the competition assay, 5 μg of the cold competitor (either purified HuCC49 or one of its variants) was added to a mixture of patient sera (collected 8 weeks post–i.v. injection with ^{177}Lu–CC49) and ^{125}I–HuCC49 and then analyzed by size–exclusion chromatography for the absence or presence of complexes. The percent inhibition of complex formation was calculated. If the variant competed with the ^{125}I–labeled MAb, and complex formation was inhibited, then the variant

still contained the immunodominant CDR. If the variant failed to inhibit complex formation, then the CDR that is no longer present in the variant is recognized by the patient and hence it is an immunogenic CDR. An example of this assay (using serum from patient LQ) is shown in FIG. 21. Panel A is the profile of the ^{125}HuCC49 in buffer only. Panel B, is the profile showing complex formation (42.9%) resulting from patient sera (LQ) incubated with ^{125}I–HuCC49. When HuCC49 is added as a competitor, there is competition for the ^{125}I–HuCC49 and a loss or absence of complexes is observed (Panel C). The same is true of a variant which still contains an immunogenic CDR (e.g., light chain CDR2 as the competitor) (Panel D). In contrast, there is either a partial (Panel F) or total retention of the complexes (Panel E), when light chain CDR1 or CDR3 variants, respectively, are the competitors.

The results are very striking, see Table 3.

Table 3: HPLC Analysis of Patient Reactivity to CDR–Replacement variants of HuCC49[a]

Competitor		Patient					
	CDR[b]	DS	DG	JG	EA	CP	LQ
None	—	33.5[c]	46.2	24.5	56.8	32.2	42.9
HuCC49	—	0	0	2.6	0.5	1.5	3.0
Hu IgG	—	46.4	59.0	25.1	63.6	ND	54.1
Light Chain	1	16.0	12.2	9.8	10.1	16.9	14.3
	2	2.7	3.4	2.7	4.4	3.0	2.4
	3	34.8	48.2	22.4	37.6	33.5	46.7
	1,2	24.6	24.5	12.6	19.4	15.7	20.2
Heavy Chain	1	10.2	3.9	3.3	7.0	5.8	3.5
	2	32.7	32.5	12.7	24.7	29.7	36.6
	3	7.3	5.1	3.7	8.2	6.7	4.6

[a] The sera from patients injected with ^{177}Lu–CC49 were tested for reactivity with variants of HuCC49 in which individual CDRs had been substituted with human sequences in both the heavy and light chains of HuCC49. Five μg of the purified CDR–replacement variants were added to a mixture of ^{125}I–HuCC49 and the patient sera and then analyzed for the presence or absence of immune complex formation.
[b] The number indicates which CDR in the HuCC49 has been replaced with a human CDR sequence.
[c] The values are the percent of complexes, the higher molecular weight species, resolved by size–exclusion chromatography.

Of the six patients analyzed, all six demonstrated reactivity with CDR3 light chain indicating that light chain CDR3 may be immunodominant in murine CC49 MAb. In the heavy chain, CDR2 appears to be dominant but not with the same level of consensus (four of the six patients show the same level of reactivity, the other two

demonstrated partial reactivity). Concordance was obtained among the six patients in regard to CDR2 of the light chain and CDR1 and CDR3 of the heavy chain, which do not appear to contribute to the immunogenicity of the MAb. This is also the case with the light chain CDR1 and, it follows, the variant with the dual substitution of CDR1 and 2 in the light chain, in which all six patients displayed a partial recognition of the variants. Partial recognition with the heavy chain CDR2 variant with two patients may be due to a loss of part but not all of the cognizant epitope, a change in the conformation or conformational epitope, or loss of amino acid residues that might stabilize the antibody:antibody interaction.

Quantitation of Patient Antibody Response

Quantitation of the HAMA or anti–variable region antibody levels in four patients was performed using HPLC analysis. The quantitation study was performed by adding either 500 ng of unlabeled BL–3 or 250 ng of HuCC49, respectively, to the mixture of patient serum and ^{125}I–HuCC49 and calculating the amount of BL–3 or HuCC49 bound in complexes.

As shown in Table 4, below, at 6 weeks, the amount of HAMA varies from patient to patient by 43–fold, while the variability of HAVRA is within 4–fold. Furthermore, the HAMA versus HAVRA levels may vary from 10 to 145–fold. Clearly, HAVRA can be detected at 3 weeks, and, not surprisingly, it does not appear to attain the same levels as HAMA. In patient EA, there is a dramatic 10–fold increase in the level of HAVRA from 6 to 8 weeks that is noteworthy.

Table 4: Quantitation of anti-CC49 variable region and anti-murine response of patients administered ^{177}Lu-CC49

		μg of Ab/ml Sera	
Patient	Post-Mab Injection	BL-3[a]	HuCC49[b]
EA	0	0	0
	3 weeks	4.1	0.3
	6 weeks	289.0	2.3
	8 weeks	314.4	21.6
CP	0	0	0
	3 weeks	16.0	0.8
	5 weeks	25.2	0.7
	6 weeks	23.2	0.7
LQ	0	0	ND
	3 weeks	4.61	0.4
	6 weeks	6.64	0.7
	8 weeks	ND	1.7
JG	0	0	0
	3 weeks	58.6	0.7
	6 weeks	47.8	2.6

Competition Radioimmunoassay

To confirm whether the HAVRA was actually an anti-idiotypic response, including internal image anti-idiotypic antibodies, to the murine MAb CC49, the sera from one patient (EA) was selected and assessed for blocking of the binding of ^{125}I-HuCC49 to BSM in a radioimmunoassay.

The immunoreactivity of the radiolabeled MAbs was assessed using bovine submaxillary mucin (BSM) immobilized on a solid support (Reacti-Gel HW65, Pierce) as a modification of the method reported by Heam et al. (1979), J. Chromatog., 185:463-470 and Schott (1992) Cancer Res., 52:6413-6417. Briefly, bovine submaxillary mucin (BSM), which is TAG-72 positive, was adsorbed to each well of a 96-well polyvinylchloride microtiter plate at 10 ng in 50µl of phosphate buffered saline (pH 7.2) as described by Horan Hand et al. (1992), Cancer Immunol. Immunother., 353:165-174. After treating the wells with 5% BSA in PBS, serial dilutions of the patient sera (25µl in 1% BSA in PBS) were added to each; ^{125}I-CC49 (38 nCi in 25 µl) was also added. Following an 18 hour incubation at 4°C, the plates were washed and the wells counted in a γ-scintillation counter. The percent inhibition was calculated and compared to that of unlabeled CC49. Human IgG (Organon Teknika, Durham, NC), which does not react with TAG-72 was included as a control antibody.

It was found that the patient sera could block the binding of ^{125}I-HuCC49 with BSM (FIG. 22) suggesting that the patient, in actuality, demonstrates an anti-idiotypic response, consisting of the internal image anti-idiotypic antibodies. Furthermore, the anti-idiotypic response was observed to increase over an eight week period. Figure 22 shows the detection of patient (EA) anti-idiotypic antibody response to murine CC49: pre-study sera from patient EA (□); sera collected at 3 weeks (A), 6 weeks (B), and 8 weeks (C).

All references cited in this disclosure are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A humanized anti-TAG-72 antibody comprising:

light chain Complementarity Determining Regions (L-CDRs), comprising L-CDR1, L-CDR2 and L-CDR3; and heavy chain Complementarity Determining Regions (H-CDRs), comprising H-CDR1, H-CDR2 and H-CDR3,

wherein L-CDR3, H-CDR1, H-CDR2 and H-CDR3 are from a non-human antibody and at least one of L-CDR1 and L-CDR2 are human antibody sequences.

2. The humanized antibody of claim 1, wherein L-CDR1 is from a human antibody.

3. The humanized antibody of claim 2, wherein L-CDR1 is from human monoclonal antibody LEN.

4. The humanized antibody of claim 1, wherein L-CDR2 from a human antibody.

5. The humanized antibody of claim 4, wherein L-CDR2 is from human monoclonal antibody LEN.

6. The humanized antibody of claim 1, wherein both L-CDR1 and L-CDR2 are human antibody sequences.

7. The humanized antibody of claim 1, wherein L-CDR1 and L-CDR2 are human antibody sequences from the same human antibody.

8. The humanized antibody of claim 7, wherein L-CDR1 and L-CDR2 are human antibody sequences from human monoclonal antibody LEN.

9. The humanized antibody of claim 6, wherein L-CDR1 and L-CDR2 are human antibody sequences from different human antibodies.

10. The humanized antibody of claim 1, wherein L–CDR3, H–CDR1, H–CDR2 and H–CDR3 are from murine monoclonal antibody CC49.

11. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein at least one amino acid of positions 60, 61, 62, or 64 in H–CDR2 is replaced with a corresponding amino acid from a human antibody.

12. The humanized antibody of claim 11, wherein the human antibody is 21/28'CL.

13. The humanized antibody of claim 11, wherein the amino acid at position 97 of L–CDR3 is replaced with a corresponding amino acid from a human antibody.

14. The humanized antibody of claim 11, wherein at least one of L–CDR1 and L–CDR2 are human antibody sequences.

15. The humanized antibody of claim 14, wherein L–CDR1 is a human antibody sequence.

16. The humanized antibody of claim 15, wherein L–CDR1 is from human monoclonal antibody LEN.

17. The humanized antibody of claim 14, wherein L–CDR2 is a human antibody sequence.

18. The humanized antibody of claim 17, wherein L–CDR2 is from human monoclonal antibody LEN.

19. The humanized antibody of claim 17, wherein both L–CDR1 and L–CDR2 are human antibody sequences.

20. The humanized antibody of claim 19, wherein L–CDR1 and L–CDR2 are human antibody sequences from the same human antibody.

21. The humanized antibody of claim 20, wherein L–CDR1 and L–CDR2 are from human monoclonal antibody LEN.

22. The humanized antibody of claim 19, wherein L–CDR1 and L–CDR2 are human antibody sequences from different human antibodies.

23. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein an amino acid at position 97 of L–CDR3 is replaced with a corresponding amino acid from a human antibody.

24. The humanized antibody of claim 23, wherein at least one amino acid of positions 60, 61, 62, or 64 in H–CDR2 is replaced with a corresponding amino acid from a human antibody.

25. The humanized antibody of claim 23, wherein at least one of L–CDR1 and L–CDR2 are human antibody sequences.

26. The humanized antibody of claim 25, wherein L–CDR1 is a human antibody sequence.

27. The humanized antibody of claim 26, wherein L–CDR1 is from human monoclonal antibody LEN.

28. The humanized antibody of claim 25, wherein L–CDR2 is a human antibody sequence.

29. The humanized antibody of claim 28, wherein L–CDR2 is from human monoclonal antibody LEN.

30. The humanized antibody of claim 25, wherein both L–CDR1 and L–CDR2 are from human antibody sequences.

31. The humanized antibody of claim 30, wherein L–CDR1 and L–CDR2 are human antibody sequences from the same human antibody.

32. The humanized antibody of claim 31, wherein L–CDR1 and L–CDR2 are from human antibody sequences from human monoclonal antibody LEN.

33. The humanized antibody of claim 30, wherein L–CDR1 and L–CDR2 are human antibody sequences from different human antibodies.

34. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein residues at positions 94 and 97 in L–CDR3 are from a non–human anti–TAG–72 antibody.

35. A humanized anti–TAG–72 antibody comprising:

light chain Complementarity Determining Regions (L–CDRs), comprising L–CDR1, L–CDR2 and L–CDR3; and heavy chain Complementarity Determining Regions (H–CDRs), comprising H–CDR1, H–CDR2 and H–CDR3,

wherein residues at positions 31, 32 and 34 in H–CDR1 are from a non–human anti–TAG–72 antibody.

36. A nucleic acid sequence expressing the humanized antibody of any of claims 1, 11, 23, 34 or 35.

37. A vector expressing the humanized antibody of any of claims 1, 11, 23, 34 or 35.

38. A composition for treatment of cancer, comprising the humanized antibody of any of claims 11, 11, 23, 34 or 35.

39. A composition for detecting cancer cells, comprising the humanized antibody of any of claims 1, 11, 23, 34 or 35.

40. A composition of for detecting cancer cells, comprising a polypeptide capable of specifically binding TAG–72, said polypeptide comprising a functional fragment of the humanized antibody of any of claims 1, 11, 23, 34 or 35.

41. The composition of claim 40, wherein the polypeptide comprises a fragment selected from the group consisting of Fv, Fab, and $F(ab')_2$.

42. A method for treating cancer comprising:

administering the humanized antibody of any of claims 1, 11, 23, 34 or 35 to a patient.

43. A method of detecting cancer cells, comprising:

contacting cells with the humanized antibody of any of claims 1, 11, 23, 34 or 35.

44. The method of claim 43, wherein the humanized antibody is labeled.

45. The method of claim 43, wherein the humanized antibody is detected using a labeled secondary antibody.

46. A method of detecting cancer cells, comprising:

contacting cells with composition comprising a polypeptide capable of specifically binding TAG−72, said polypeptide comprising a functional fragment of the humanized antibody of any of claims 11, 11, 23, 34 or 35.

47. The method of claim 46, wherein the polypeptide comprises a fragment selected from the group consisting of Fv, Fab, and $F(ab')_2$.

FIG. 1

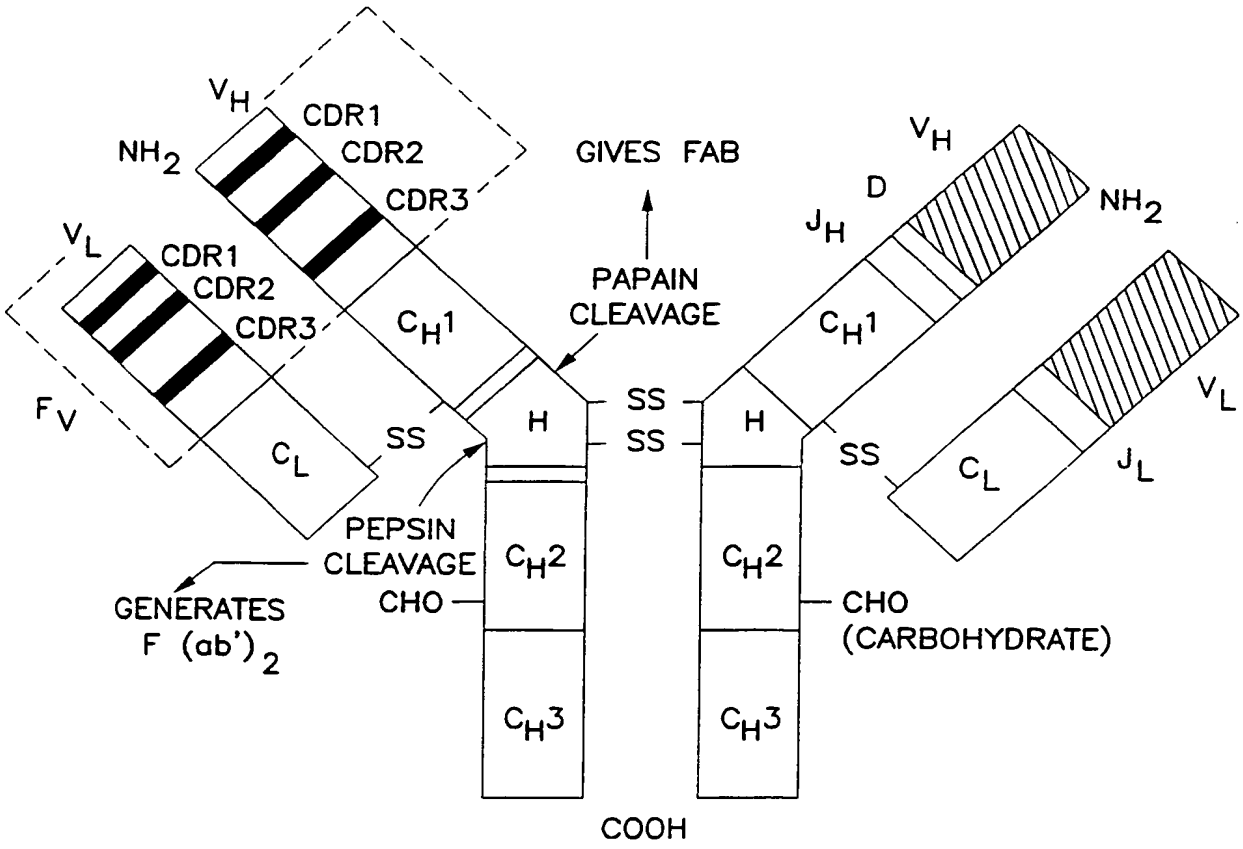

V_H
CDR1
CDR2
CDR3
NH_2
V_L
CDR1
CDR2
CDR3
F_V
C_L
C_H1
SS
H
PEPSIN
CLEAVAGE
GENERATES
F (ab')$_2$
CHO
C_H2
C_H3
GIVES FAB
PAPAIN
CLEAVAGE
SS
SS
V_H
D
J_H
NH_2
C_H1
H
SS
C_L
V_L
J_L
C_H2
CHO
(CARBOHYDRATE)
C_H3
COOH

FIG. 2

Light chain

CDR 1	24	25	26	27	a	b	c	d	e	f	28	29	30	31	32	33	34
HuCC49	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Tyr	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Ala
LEN	Lys	Ser	Ser	Gln	Ser	*Val*	Leu	Tyr	Ser	*Ser*	Asn	*Ser*	Lys	Asn	Tyr	Leu	Ala

CDR 2	50	51	52	53	54	55	56
HuCC49	Trp	Ala	Ser	Ala	Arg	Glu	Ser
LEN	Trp	Ala	Ser	*Thr*	Arg	Glu	Ser

CDR 3	89	90	91	92	93	94	95	96	97
HuCC49	Gln	Gln	Tyr	Tyr	Ser	Tyr	Pro	Leu	Thr
LEN	Gln	Gln	Tyr	Tyr	Ser	*Thr*	Pro	*Tyr*	*Ser*

Heavy chain

CDR 1	31	32	33	34	35
HuCC49	Asp	His	Ala	Ile	His
21/28'CL	*Ser*	*Tyr*	Ala	*Met*	Hls

CDR 2	50	51	52	a	53	54	55	56	57	58	59	60	61	62	63	64	65
HuCC49	Tyr	Phe	Ser	Pro	Gly	Asn	Asp	Asp	Phe	Lys	Tyr	Asn	Glu	Arg	Phe	Lys	Gly
21/28'CL	*Trp*	*Ile*	*Asn*	*Ala*	Gly	Asn	*Gly*	*Asn*	*Thr*	Lys	Asn	*Ser*	*Gln*	*Lys*	Phe	*Gln*	Gly

CDR 3	95	96	97	98	99	100	a	b	101	102
HuCC49	Ser	Leu	Asn	Met	Ala	–	–	–	–	Tyr
21/28'CL	*Gly*	*Gly*	*Tyr*	*Tyr*	*Gly*	*Ser*	*Gly*	*Ser*	*Asn*	Tyr

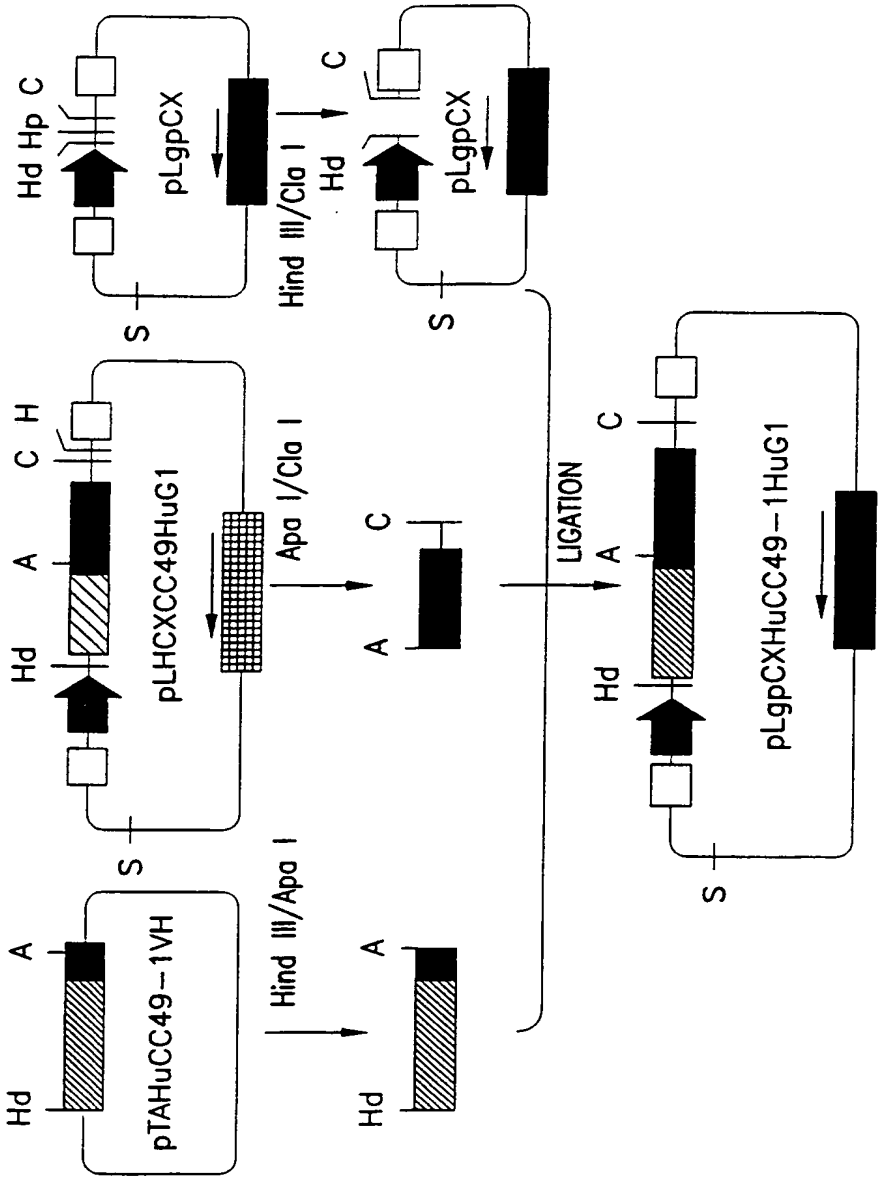
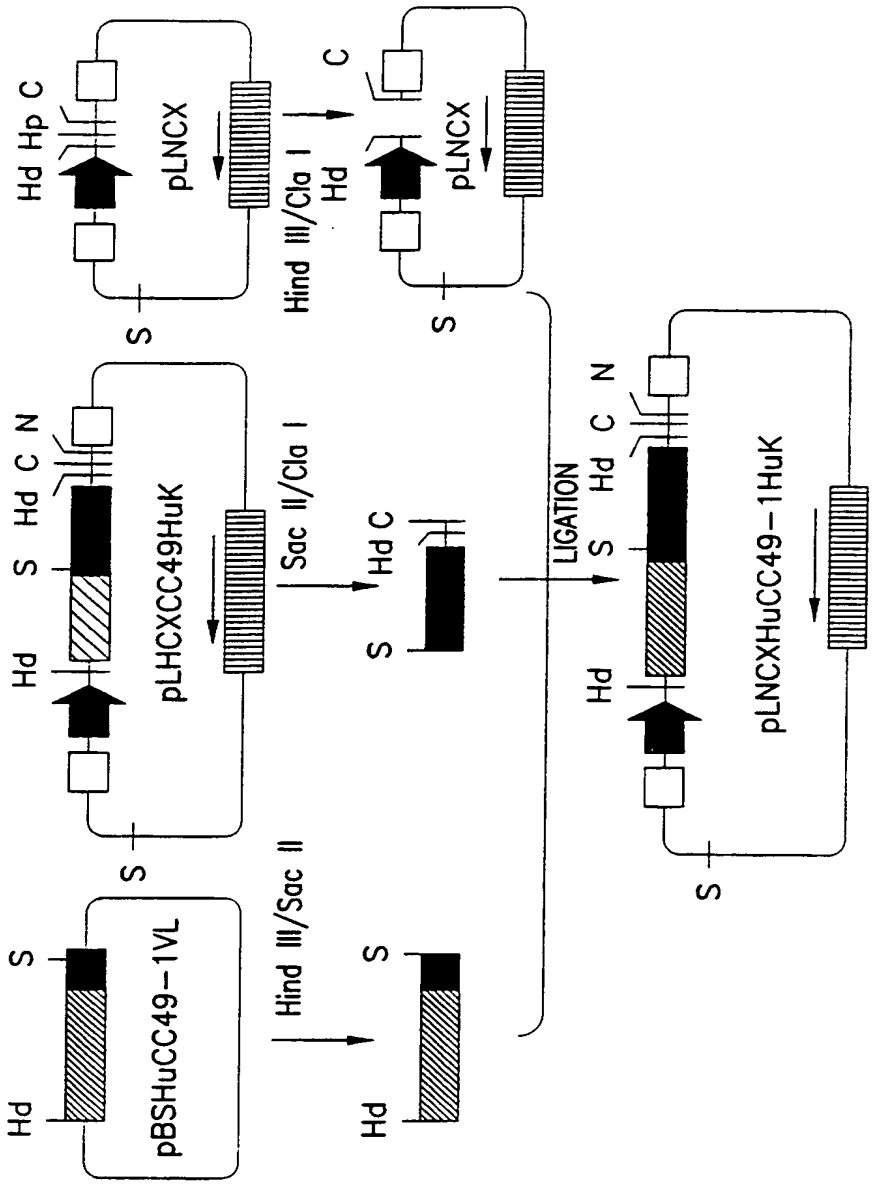

Hd Hp C
pLgpCX
S
Hind III/Cla I
C
Hd
pLgpCX
S
C H
A
Hd
pLHCXCC49HuG1
S
Apa I/Cla I
C
A
LIGATION
C
A
Hd
pLgpCXHuCC49-1HuG1
S
A
Hd
pTAHuCC49-1VH
Hind III/Apa I
A
Hd

FIG. 3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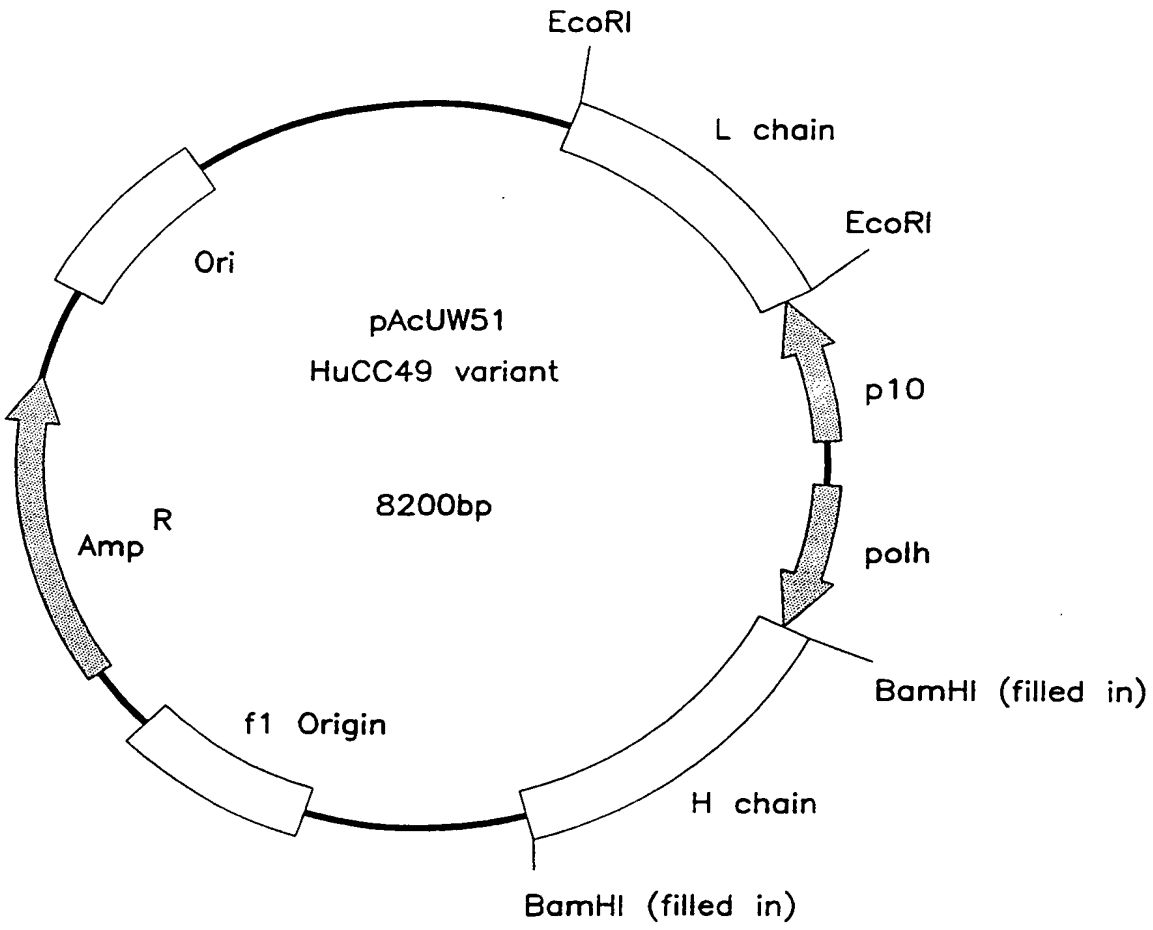
pLNCX
S
Hind III/Cla I
C
Hd
pLNCX
S
S Hd C N
Hd
pLHCXCC49HuK
S
Sac II/Cla I
Hd C
S
LIGATION
S Hd C N
Hd
pLNCXHuCC49-1HuK
S
S
Hd
pBSHuCC49-1VL
Hind III/Sac II
S
Hd

FIG. 4

FIG. 5


EcoRI
L chain
EcoRI
Ori
pAcUW51
HuCC49 variant
p10
8200bp
Amp R
polh
BamHI (filled in)
f1 Origin
H chain
BamHI (filled in)

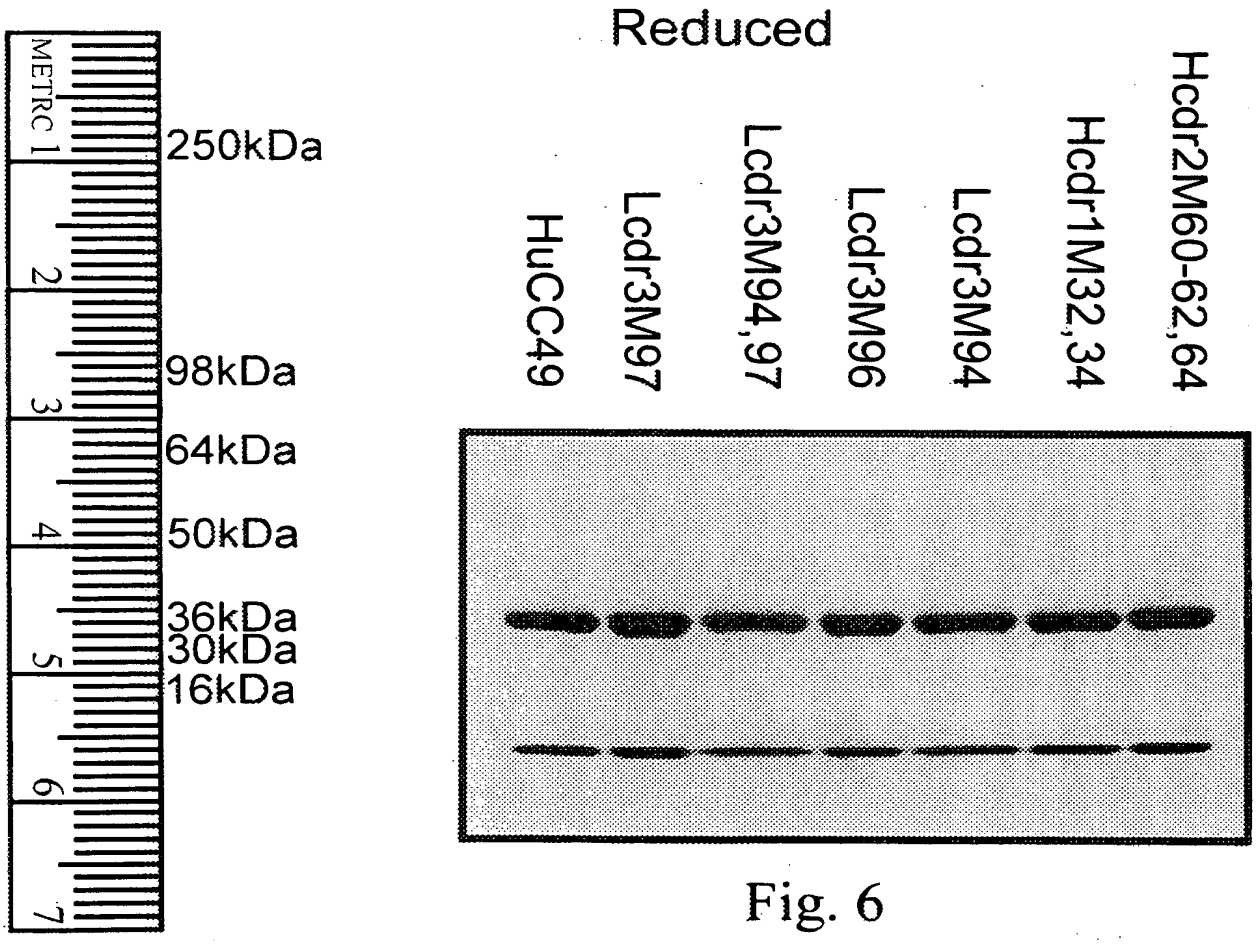

Reduced
Hcdr2M60-62,64
Hcdr1M32,34
Lcdr3M94
Lcdr3M96
Lcdr3M94,97
Lcdr3M97
HuCC49
METRC
250kDa
98kDa
64kDa
50kDa
36kDa
30kDa
16kDa

Fig. 6

FIG. 7

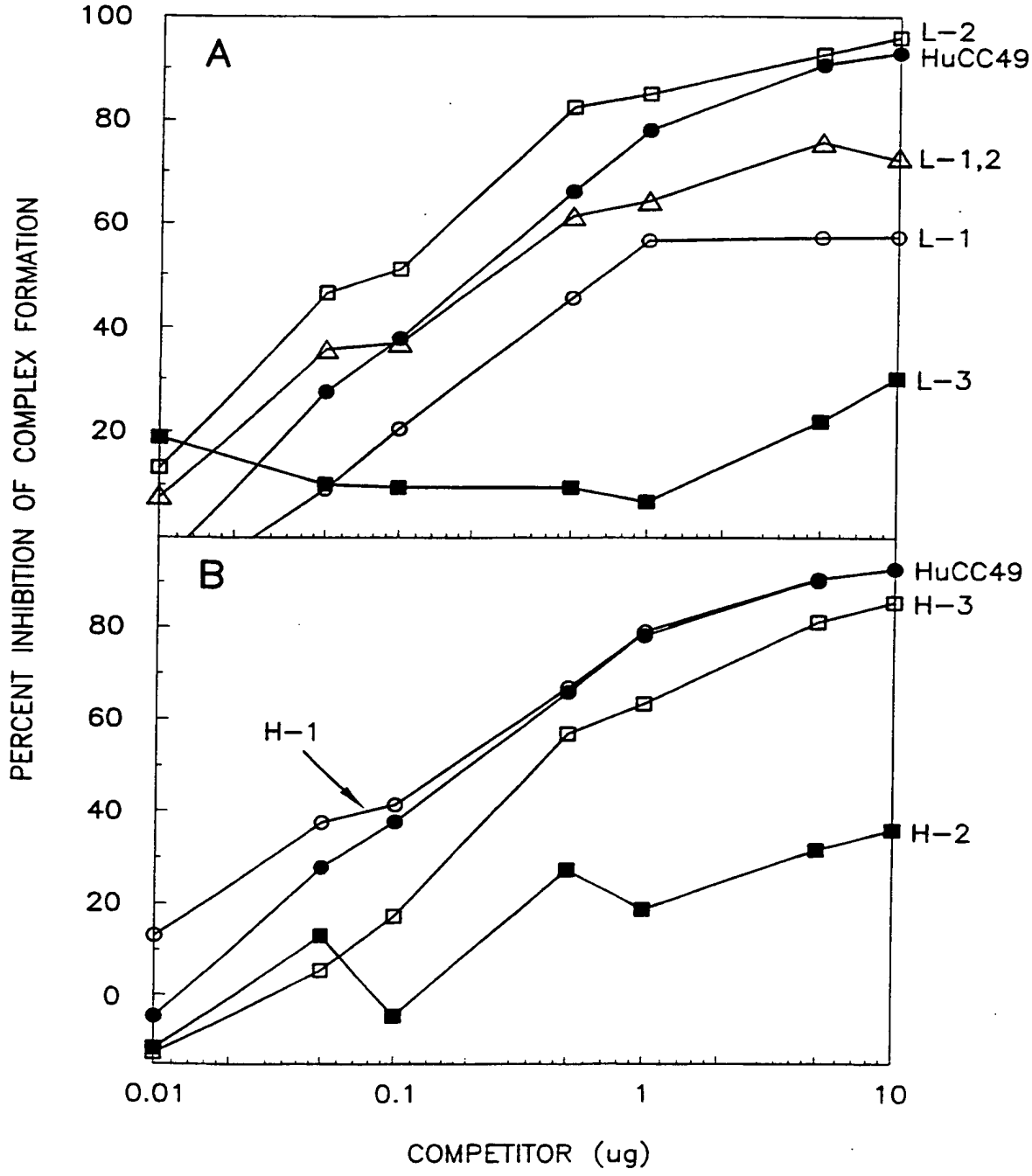

A
B
L-2
HuCC49
L-1,2
L-1
L-3
HuCC49
H-3
H-1
H-2
PERCENT INHIBITION OF COMPLEX FORMATION
COMPETITOR (ug)
100
80
60
40
20
0
0.01
0.1
1
10

FIG. 8

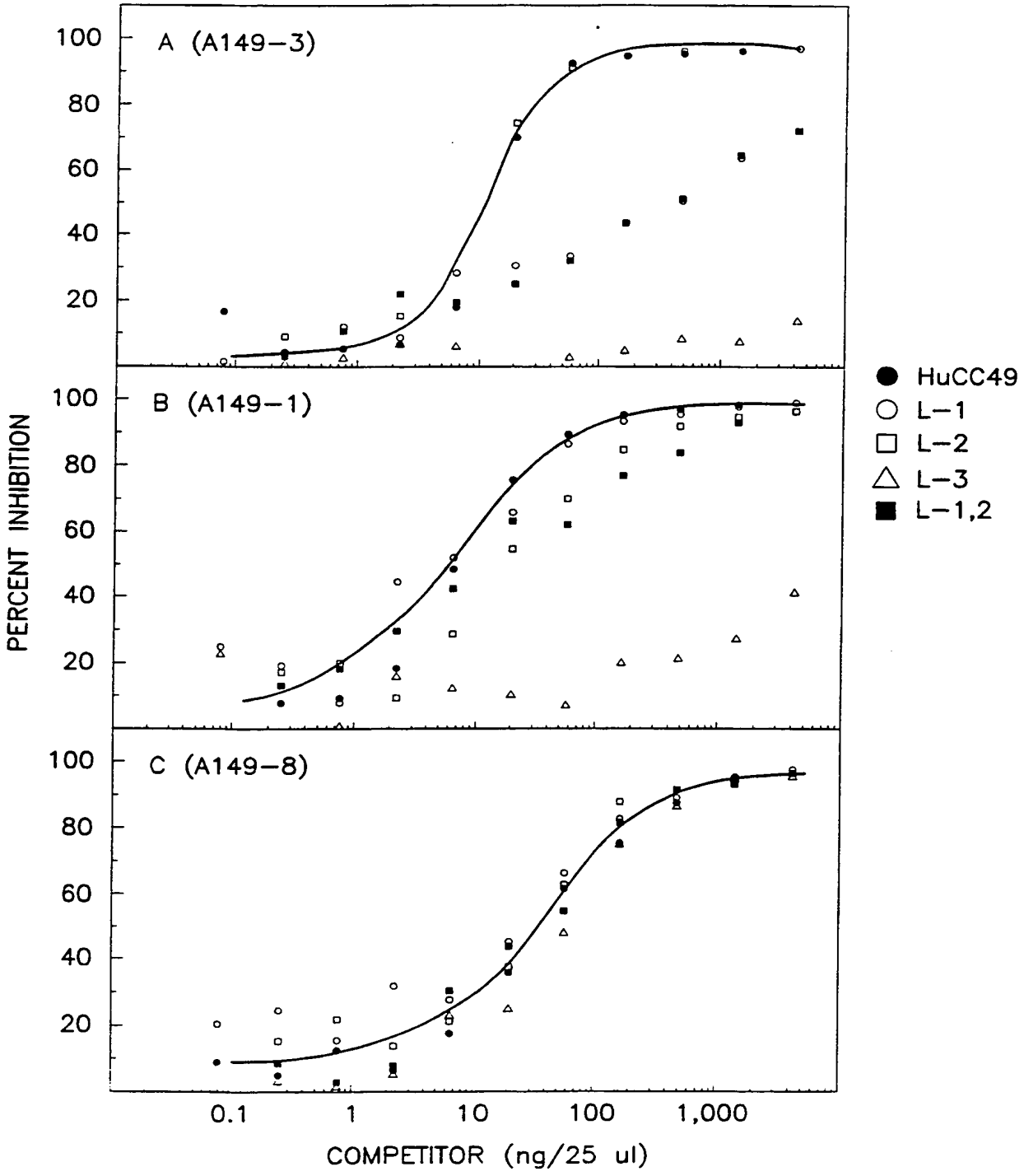

A (A149-3)
B (A149-1)
C (A149-8)
PERCENT INHIBITION
100
80
60
40
20
0.1
1
10
100
1,000
COMPETITOR (ng/25 ul)
HuCC49
L-1
L-2
L-3
L-1,2

FIG. 9

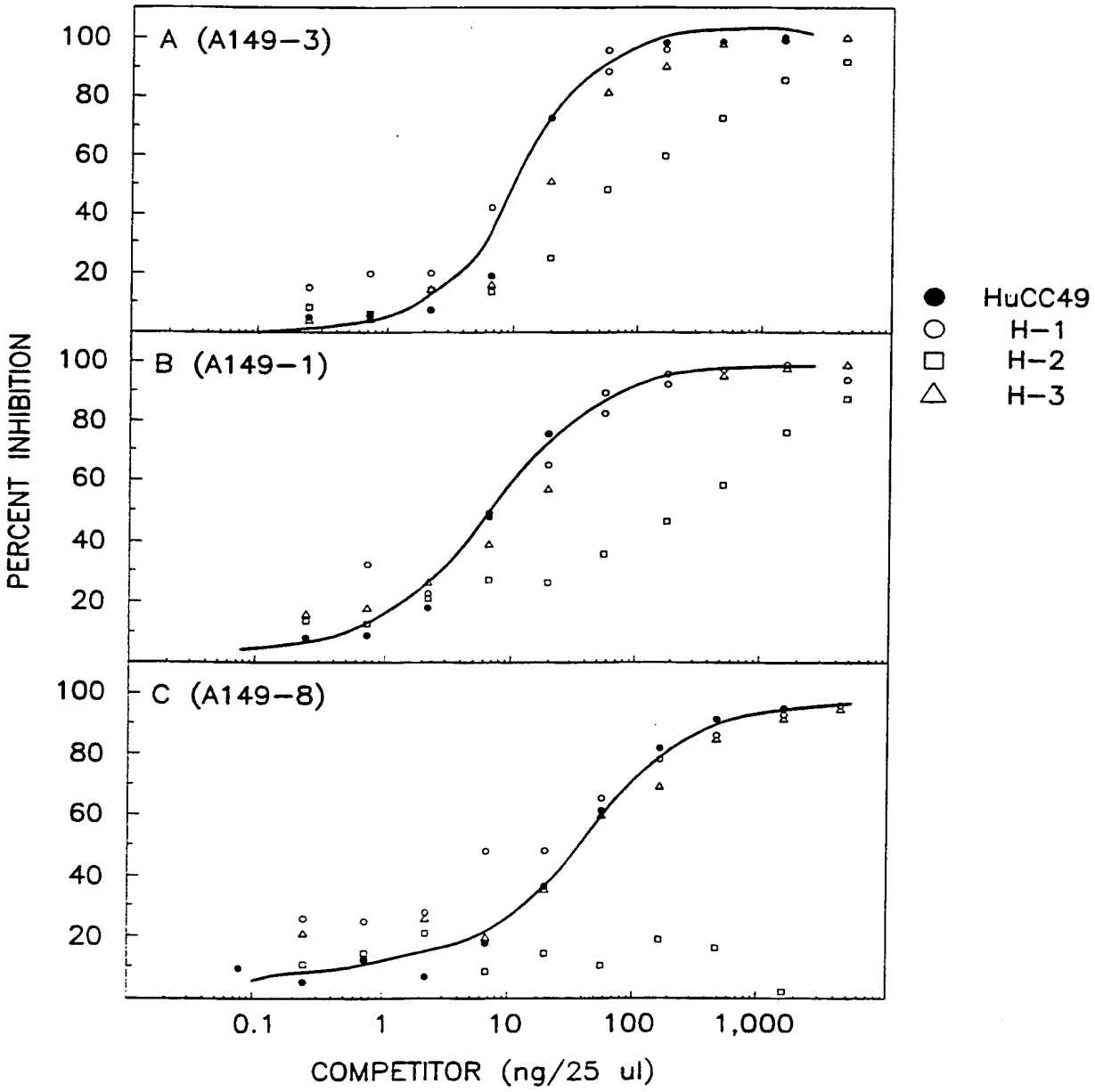

A (A149-3)
B (A149-1)
C (A149-8)
PERCENT INHIBITION
100
80
60
40
20
0.1
1
10
100
1,000
COMPETITOR (ng/25 ul)
HuCC49
H-1
H-2
H-3

FIG. 10

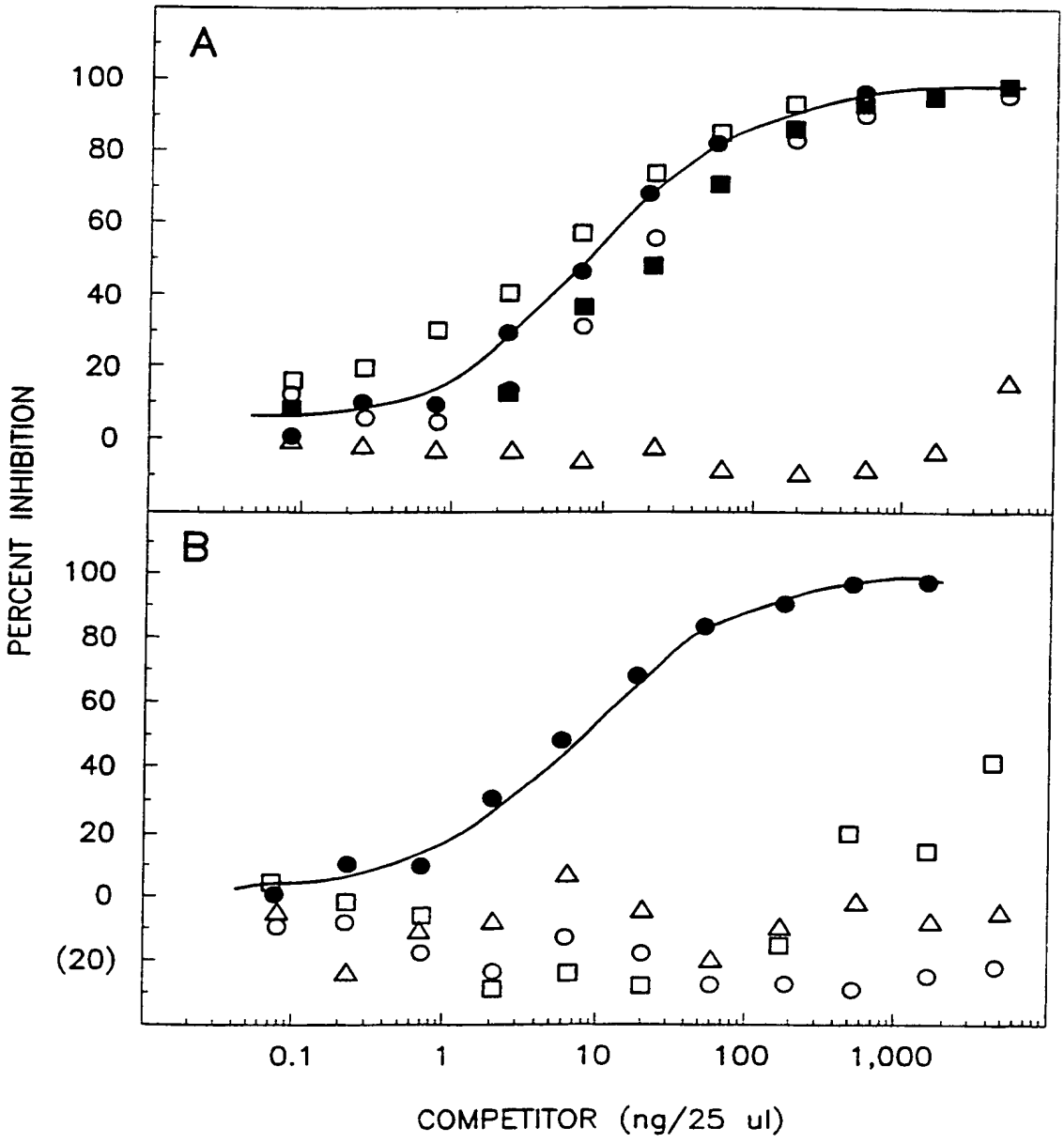
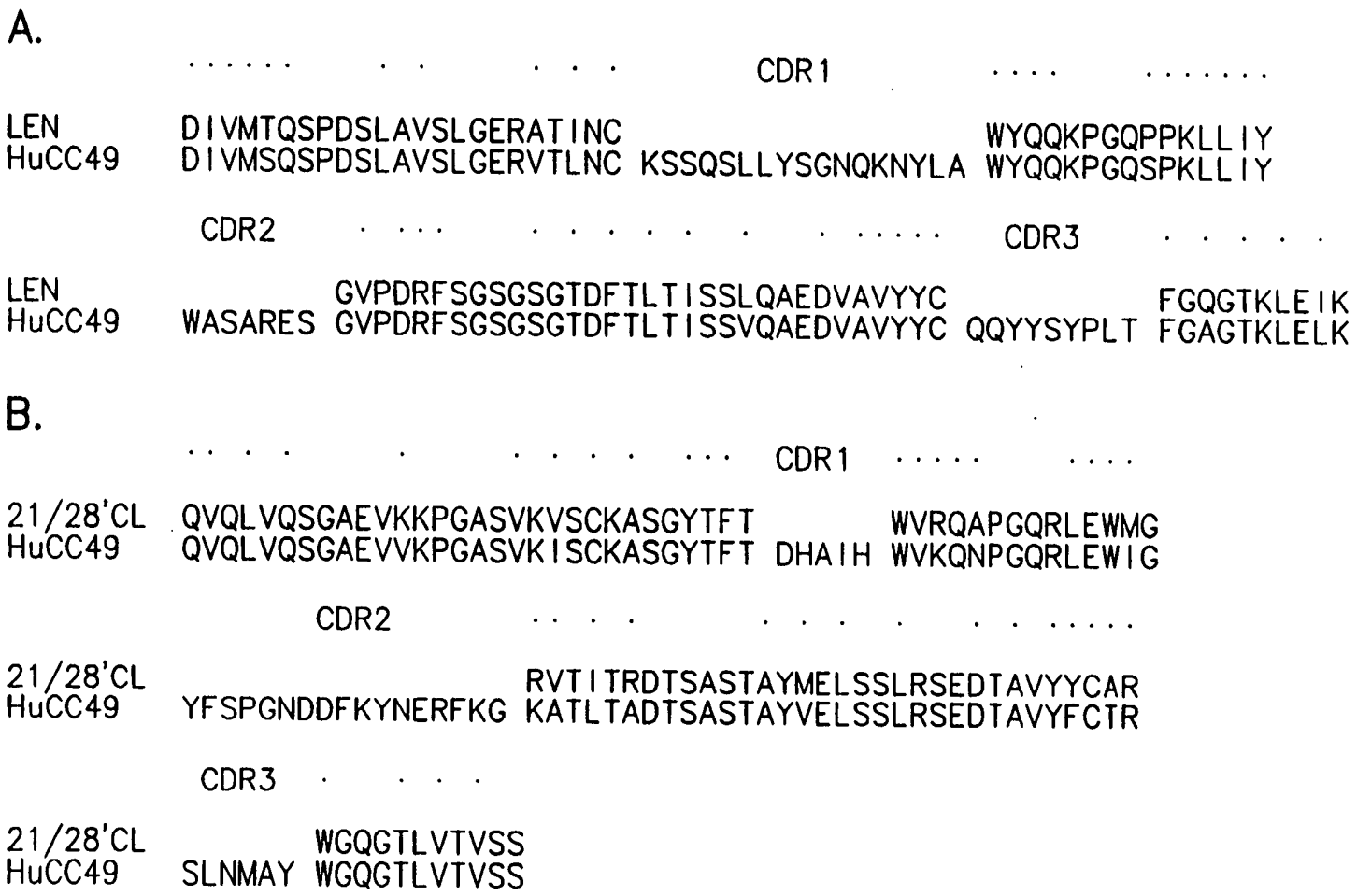
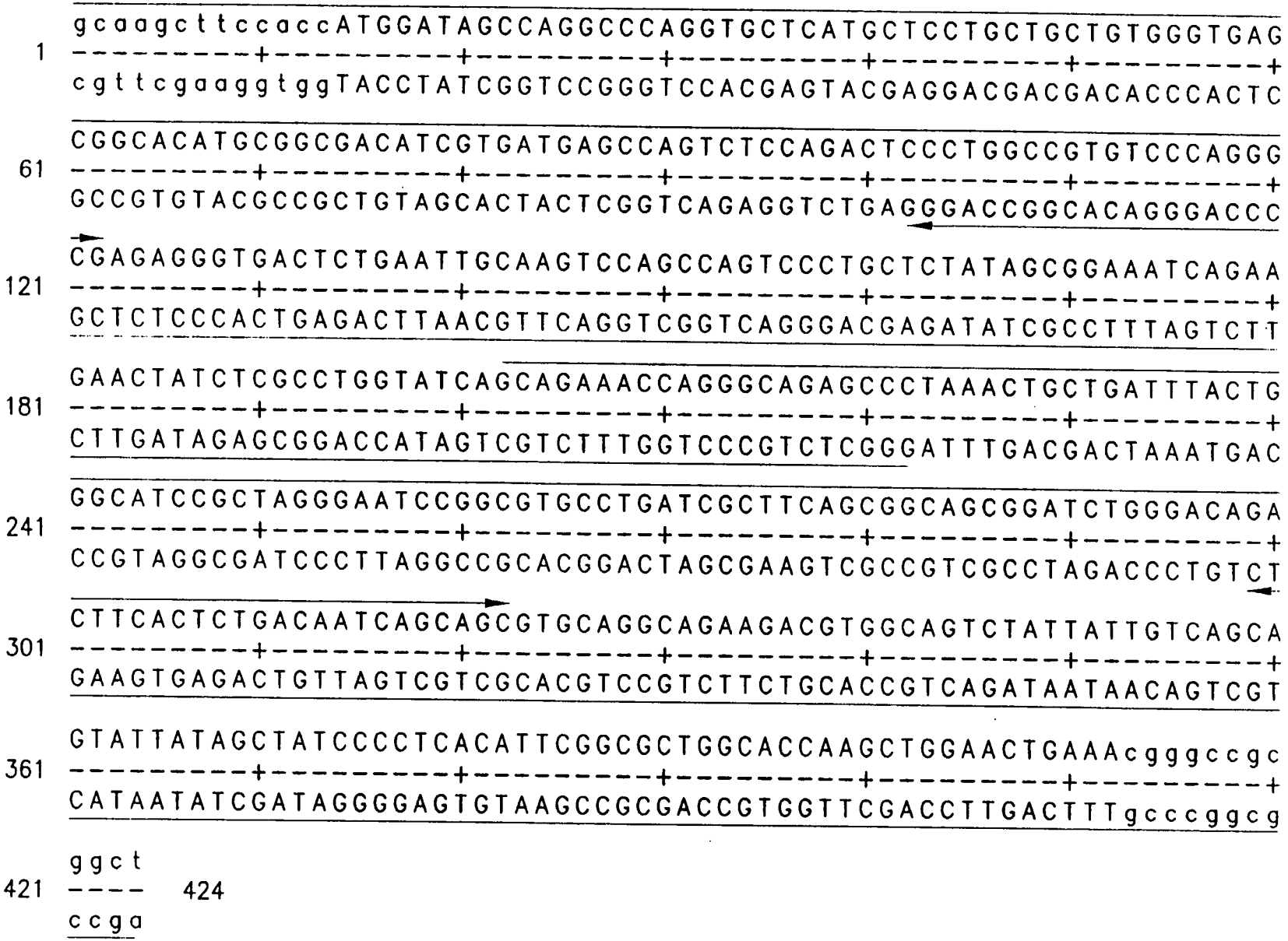

A
B
100
80
60
40
20
0
(20)
PERCENT INHIBITION
0.1
1
10
100
1,000
COMPETITOR (ng/25 ul)

FIG. 11



A.

FIG. 12A



B.

FIG. 12B

```
     ctaagcttccaccATGGAGTGGTCCTGGGTCTTCCTCTTCCTCCTGCTGTGGGTGAG
  1  ----------+---------+---------+---------+---------+---------+  60
     gattcgaaggtggTACCTCACCAGGACCCAGAAGGAGAAGGAGGACGACACCCACTC

     AGTGCACTCCCAGGTCCAGCTGGTGCAGTCCGGCGCTGAGTCCCTGGCCGTGTCCCAGGG
 61  ----------+---------+---------+---------+---------+---------+  120
     TCACGTGAGGGTCCAGGTCGACCACGTCAGGCCGCGACTCAGGGACCGGCACAGGGACCC

     CGTGAAGATTTCCTGCAAGGCAAGCGGCTACACCTTCACTCTCTATAGCGGAAATCAGAA
121  ----------+---------+---------+---------+---------+---------+  180
     GCACTTCTAAAGGACGTTCCGTTCGCCGATGTGGAAGTGAGAGATATCGCCTTTAGTCTT

     GAAACAGAATCCTGGACAGCGCCTGGAGTGGATTGGATATTTCTCTCCCGGAAACGATGA
181  ----------+---------+---------+---------+---------+---------+  240
     CTTTGTCTTAGGACCTGTCGCGGACCTCACCTAACCTATAAAGAGAGGGCCTTTGCTACT

     TTTTAAGTACAATGAGAGGTTCAAGGGCAAGGCCACACTGACTGCAGACACATCTGCCAG
241  ----------+---------+---------+---------+---------+---------+  300
     AAAATTCATGTTACTCTCCAAGTTCCCGTTCCGGTGTGACTGACGTCTGTGTAGACGGTC

     CACTGCCTACGTGGAGCTCTCCAGCCTGAGATCCGAGGATACTGCAGTGTACTTCTGCAC
301  ----------+---------+---------+---------+---------+---------+  360
     GTGACGGATGCACCTCGAGAGGTCGGACTCTAGGCTCCTATGACGTCACATGAAGACGTG

     AAGATCCCTGAATATGGCCTACTGGGGACAGGGAACCCTGGTCACCGTCTCCAGCgccaa
361  ----------+---------+---------+---------+---------+---------+  420
     TTCTAGGGACTTATACCGGATGACCCCTGTCCCTTGGGACCAGTGGCAGAGGTCGcggtt

     aactacgggcccat
421  ----------+----  434
     ttgatgcccgggta
```

FIG. 13

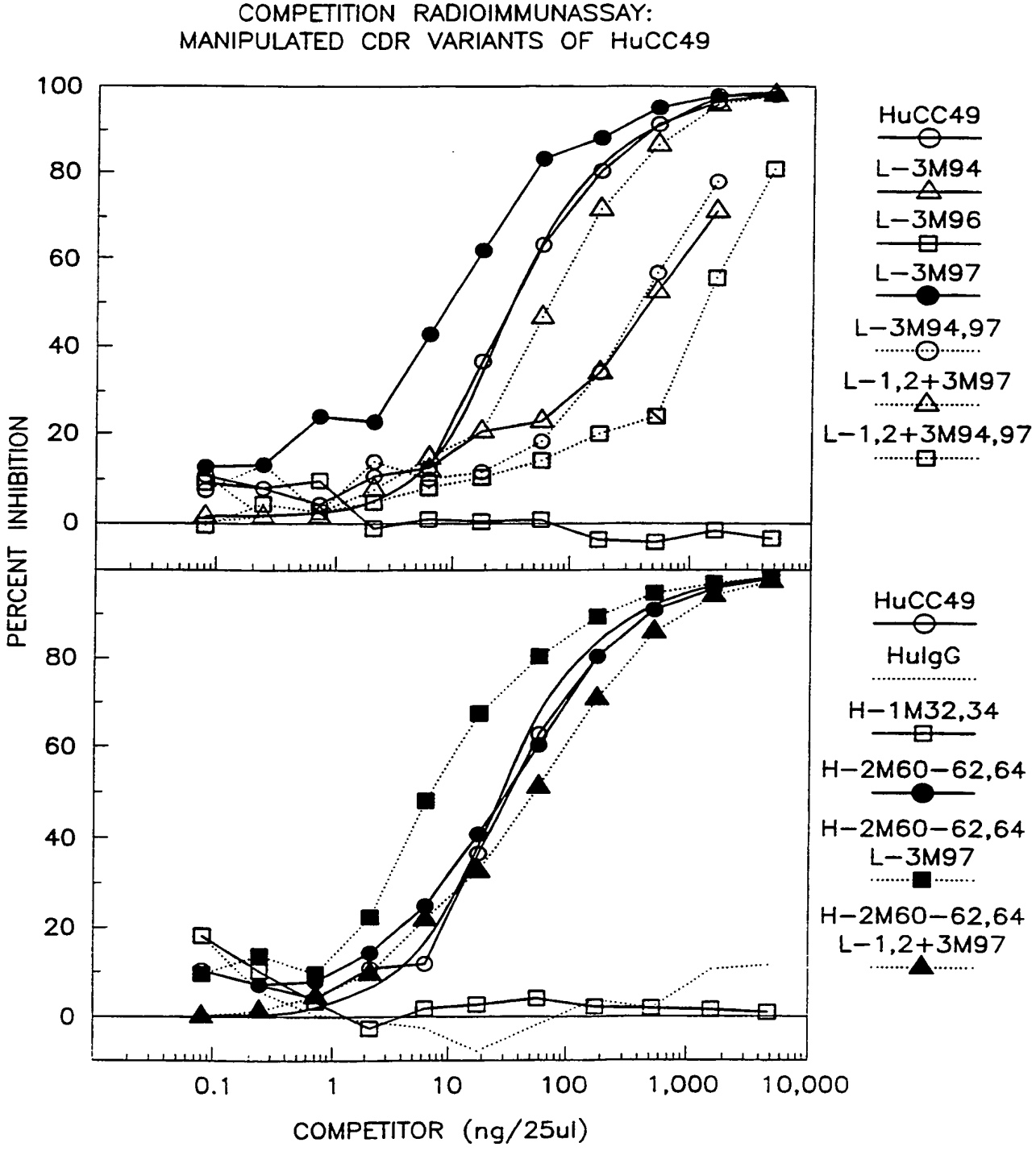

COMPETITION RADIOIMMUNASSAY:
MANIPULATED CDR VARIANTS OF HuCC49
PERCENT INHIBITION
100
80
60
40
20
0
HuCC49
L-3M94
L-3M96
L-3M97
L-3M94,97
L-1,2+3M97
L-1,2+3M94,97
HuCC49
HuIgG
H-1M32,34
H-2M60-62,64
H-2M60-62,64
L-3M97
H-2M60-62,64
L-1,2+3M97
0.1
1
10
100
1,000
10,000
COMPETITOR (ng/25ul)

FIG. 14

HPLC ANALYSIS OF PATIENT REACTIVITY TO CDR SUBSTITUTION VARIANTS OF HuCC49

COMPETITOR			PATIENTS			
	CDR SUBSTITUTIONS	ANTIGEN BINDING	DG	CP	EA	DS
NONE	---		46.2[b]	32.2	56.8	33.5
HuCC49	---	+++	0	1.5	0.5	0
HuIgG	---	–	59.0	N.D.	63.6	46.4
LIGHT	L3M94	+/–	30.2	20.3	16.4	28.9
	L3M96	–	39.2	31.1	42.9	35.2
	L3M97	+++	0.6	1.3	0.7	2.4
	L3M94,97	+/–	26.5	18.2	18.6	25.6
	L1,2+3M97	++	21.3	17.6	23.8	17.1
	L1,2+3M94,97	+	53.2	38.1	44.2	37.3
HEAVY	1M32,34	–	1.4	5.5	3.8	0.7
	2M60–62,64	++	24.4	17.9	21.8	16.5
BOTH	L3M97 H2M60–62,64	++++	13.0	16.1	3.9	20.1
	L1,2+3M97 H2M60–62,64	++	33.0	30.7	24.9	32.1

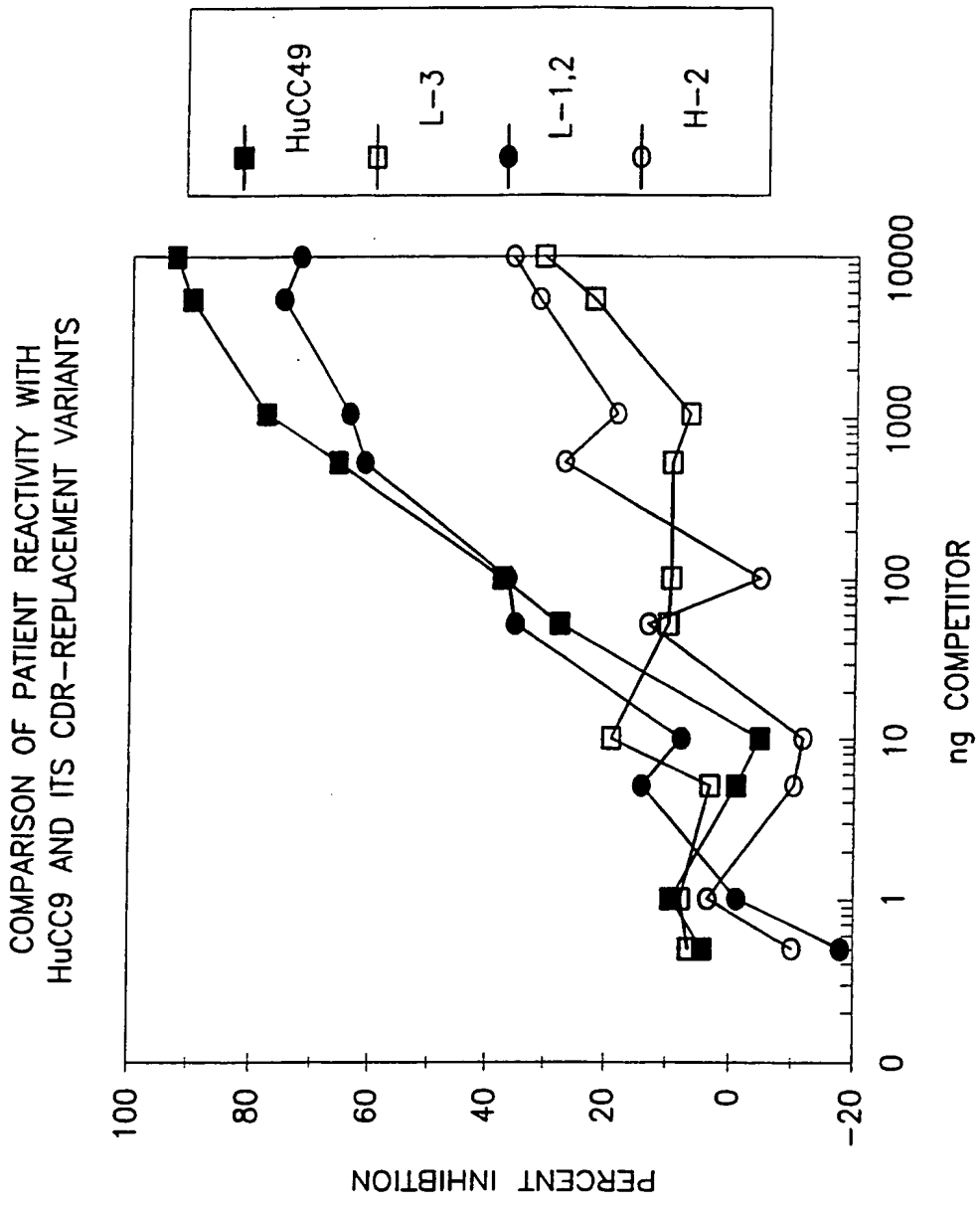

COMPARISON OF PATIENT REACTIVITY WITH
HuCC9 AND ITS CDR-REPLACEMENT VARIANTS
HuCC49
L-3
L-1,2
H-2
PERCENT INHIBITION
100
80
60
40
20
0
-20
0
1
10
100
1000
10000
ng COMPETITOR

FIG. 15

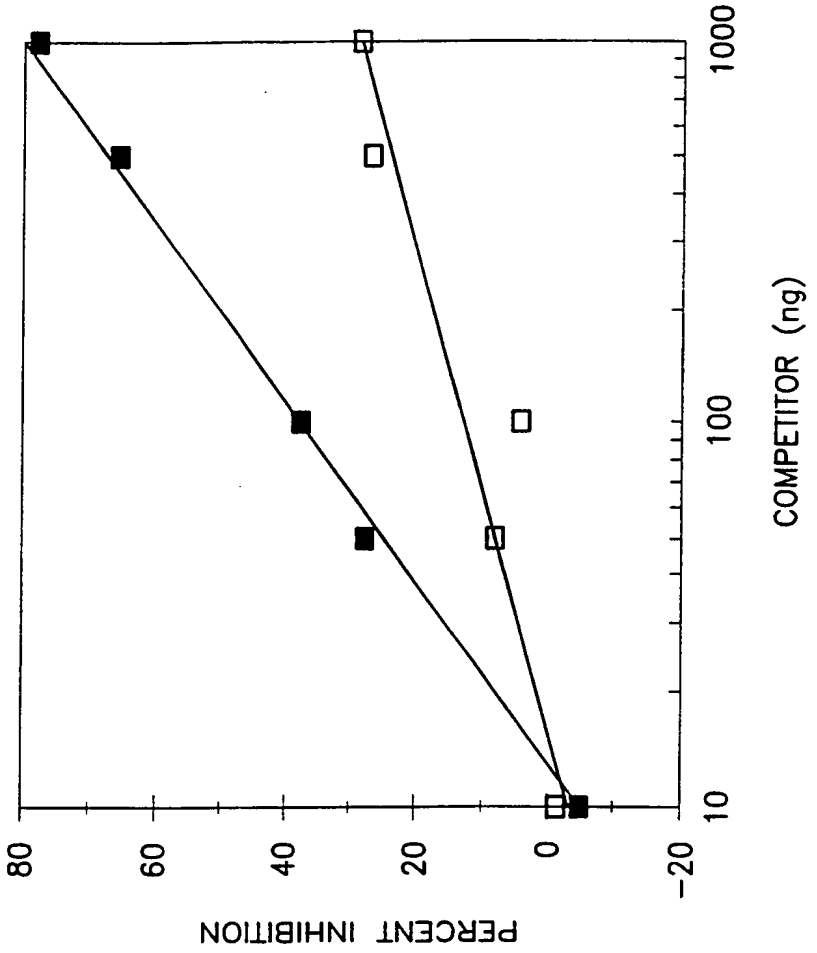


FIG. 16

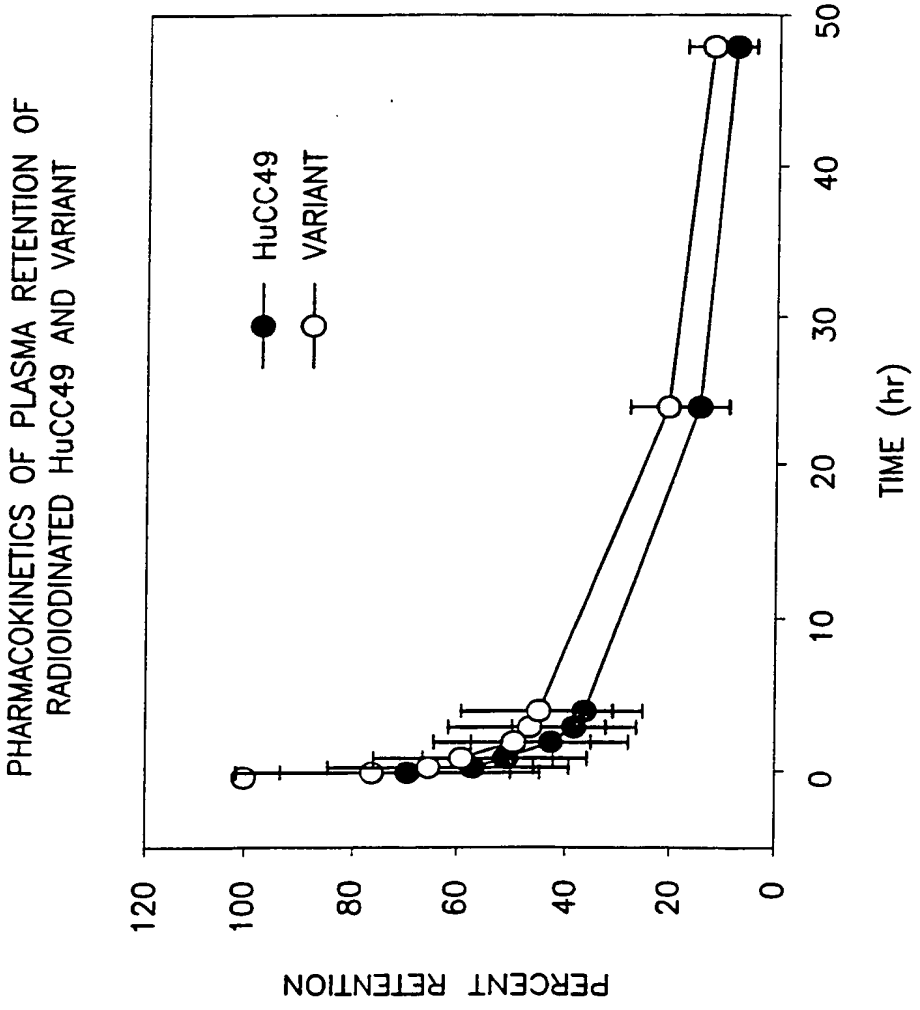

PHARMACOKINETICS OF PLASMA RETENTION OF
RADIOIODINATED HuCC49 AND VARIANT
HuCC49
VARIANT
PERCENT RETENTION
120
100
80
60
40
20
0
0
10
20
30
40
50
TIME (hr)

FIG. 17

FIG. 18

BIODISTRIDUTION OF I.V. ADMINISTERED RADIOLABELED HuCC49 AND VARIANT IN ATHYMIC MICE BEARING LS-174T HUMAN COLON CARCINOMA XENOGRAFTS: PERCENT OF INJECTED DOSE/GRAM

		TIMEPOINTS (hr)				
ANTIBODY	ORGAN	24	48	72	120	168
VARIANT	TUMOR	15.83	23.75	21.01	17.74	9.21
	BLOOD	6.35	4.93	4.88	2.19	0.63
	LIVER	3.39	2.14	1.46	0.91	0.32
	SPLEEN	5.90	6.04	2.55	2.43	3.96
	KIDNEY	2.52	1.27	1.00	0.77	0.36
	LUNG	3.22	2.57	2.50	1.12	0.36
HuCC49	TUMOR	11.86	17.59	15.31	13.75	5.24
	BLOOD	4.17	2.94	2.85	1.29	0.18
	LIVER	4.77	3.05	1.41	0.70	0.12
	SPLEEN	6.41	7.47	2.28	2.00	0.46
	KIDNEY	1.86	0.92	0.70	0.57	0.14
	LUNG	2.17	1.58	1.46	0.68	0.12

FIG. 19

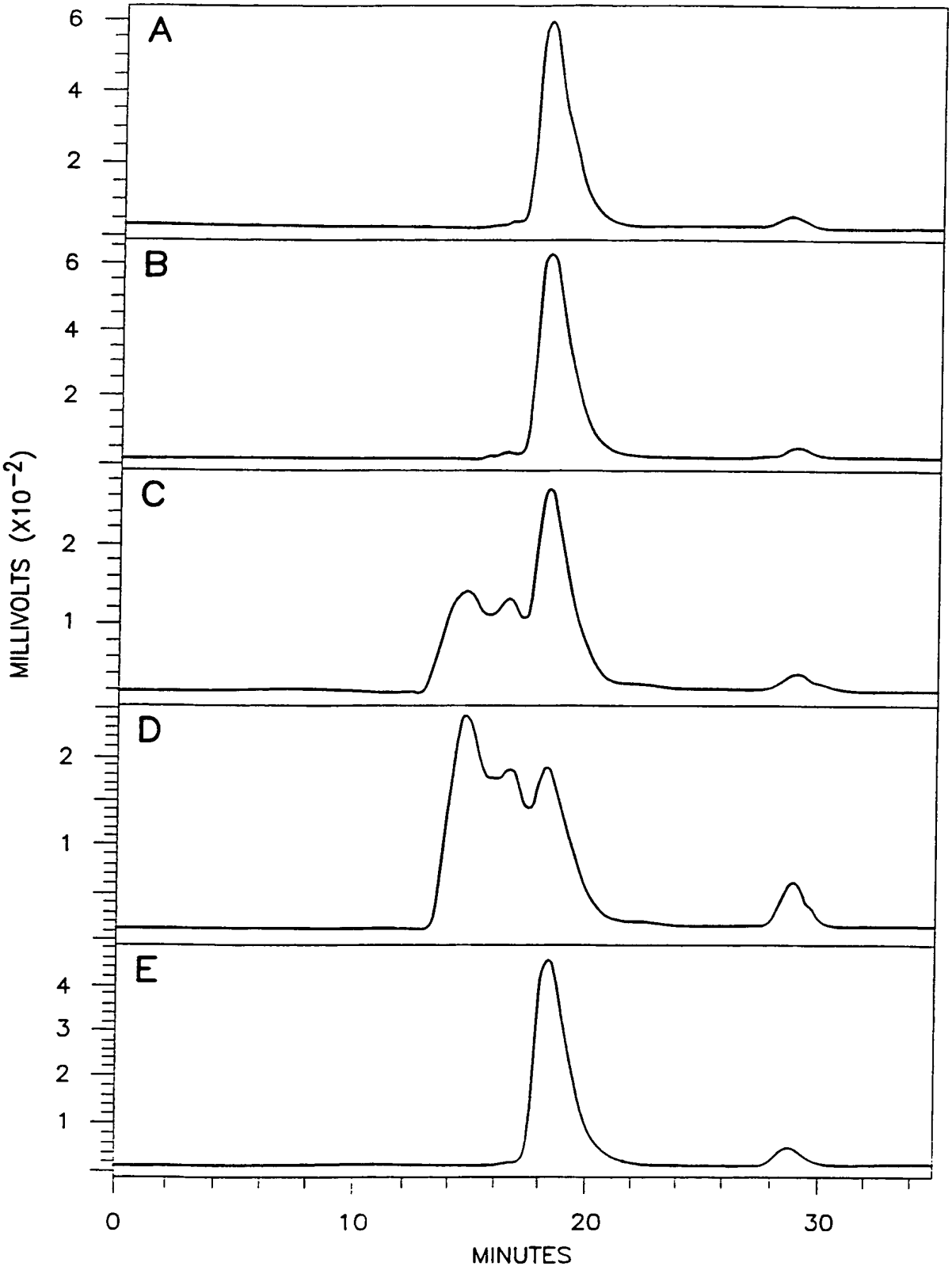
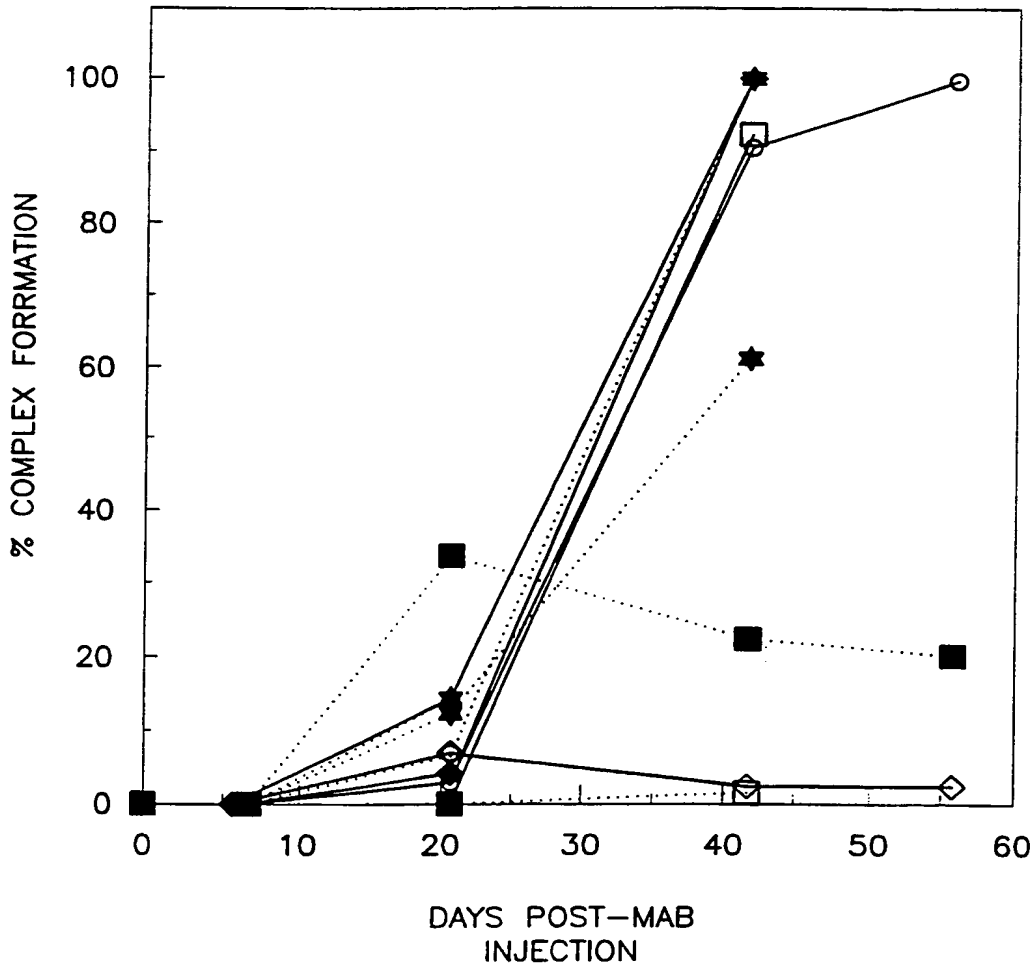


FIG. 20


% COMPLEX FORMATION
100
80
60
40
20
0
0
10
20
30
40
50
60
DAYS POST—MAB INJECTION

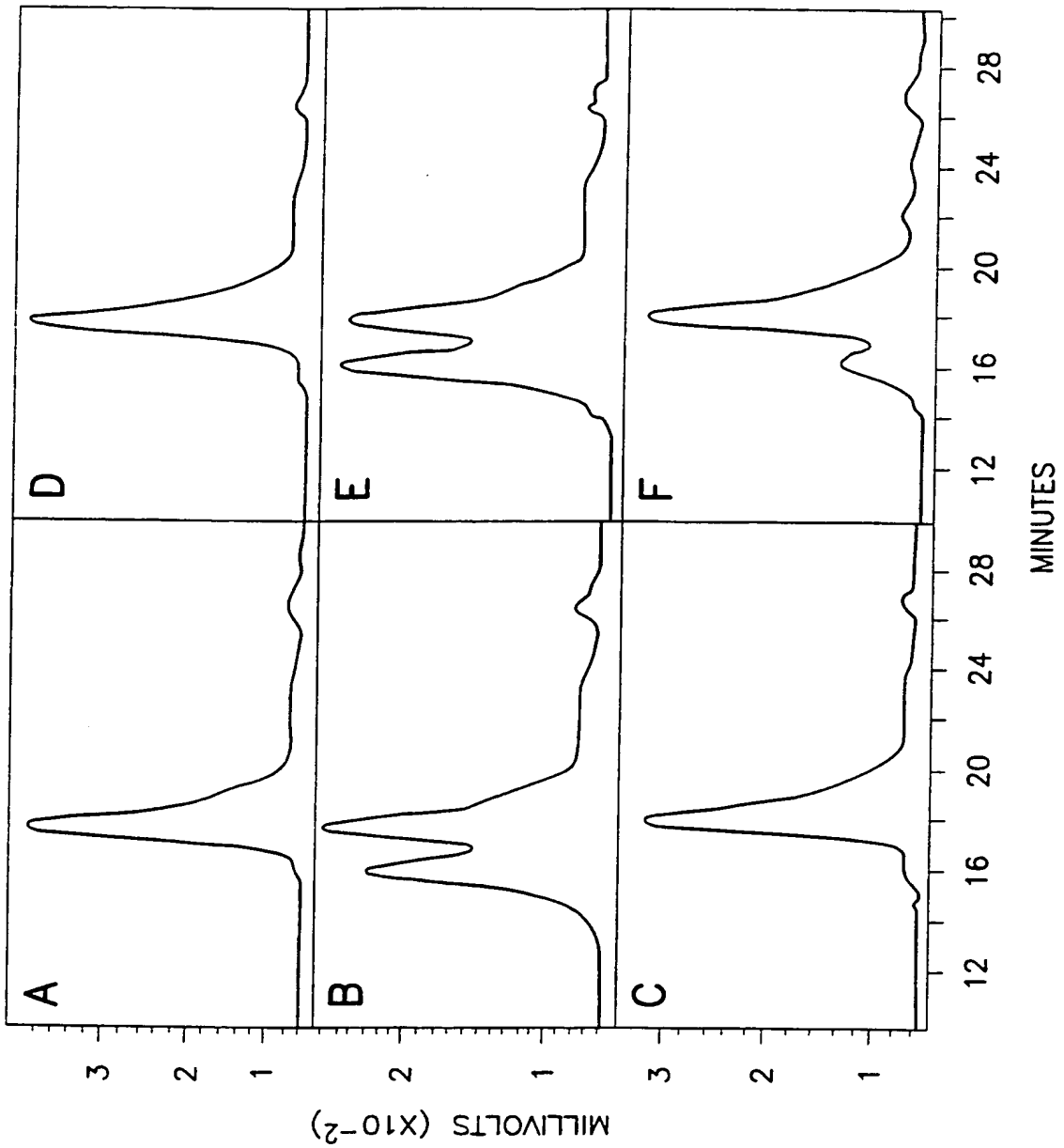


FIG. 21

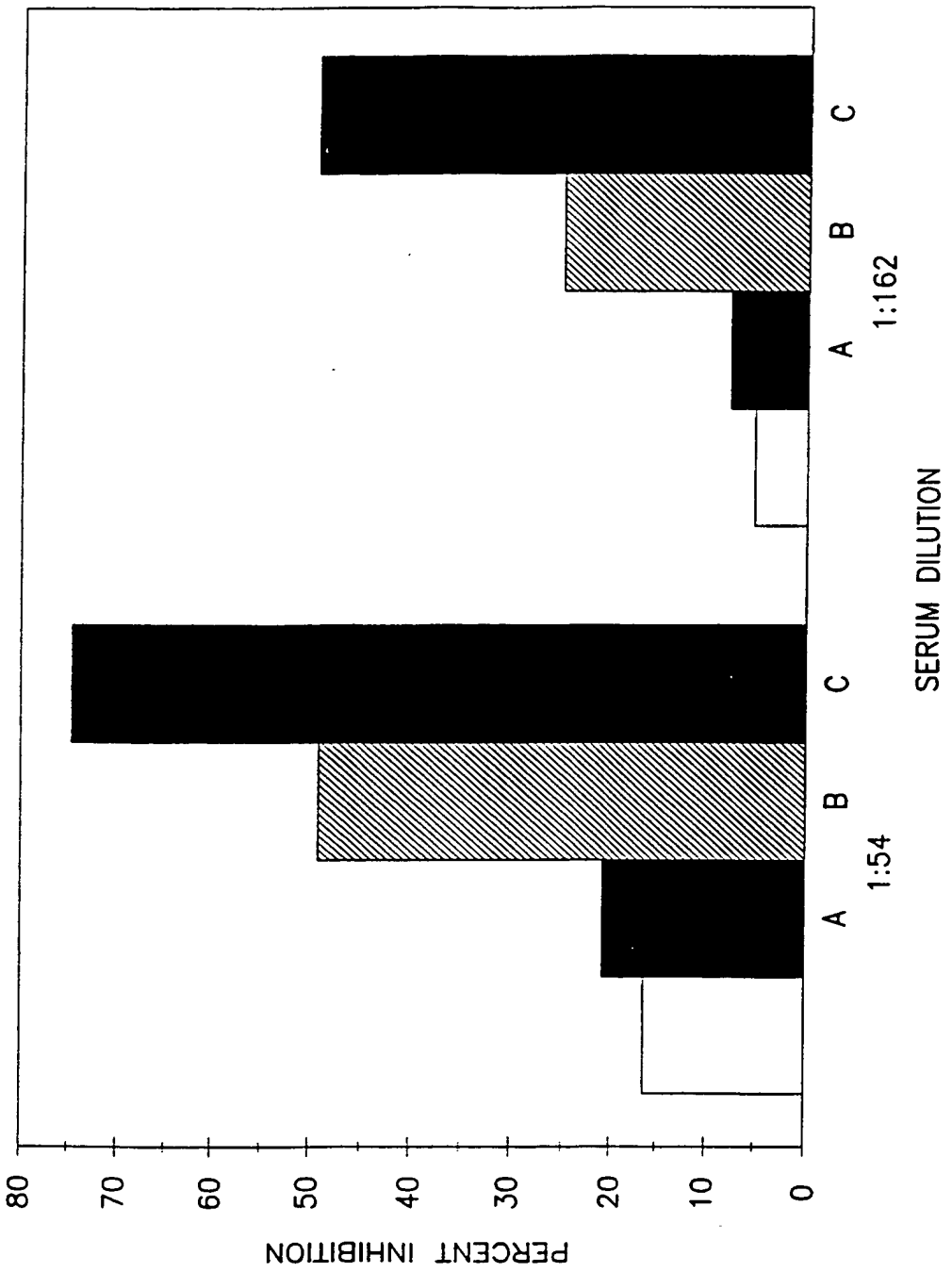

PERCENT INHIBITION
80
70
60
50
40
30
20
10
0
A
B
C
1:54
A
B
C
1:162
SERUM DILUTION

FIG. 22

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/25552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/85 C12N15/62 C12N5/10 C07K16/30 C07K16/46
A61K51/10 A61P35/00 G01N33/574 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y SHA ET AL: "A heavy-chain grafted antibody that recognizes the tumor-associated TAG72 antigen" CANCER BIOTHERAPY, vol. 9, no. 4, 1 January 1994 (1994-01-01), pages 341-349, XP002079337 abstract page 342, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 346, left-hand column, paragraph 2 -page 347, right-hand column, paragraph 1 ---	1-47
X	WO 97 26010 A (SMITHKLINE BEECHAM CORP., USA;UNIVERSITY OF VERMONT AND STATE AGRICULT) 24 July 1997 (1997-07-24) page 9, line 28 -page 10, line 10 page 21, line 25 -page 22, line 13 --- -/--	1,2,4,6, 7,9, 36-41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: "Complementarity determining region residues aspartic acid at H55, serine at H95 and tyrosines at H97 and L96 play important roles in the B72.3 antibody-TAG72 antigen interaction." retrieved from STN Database accession no. 97015918 XP002134981 abstract & PROTEIN ENGINEERING, (1996 JUN) 9 (6) 539-43. , ---	23,36-47
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US XIANG J ET AL: "The tyrosine residue at position 97 in the VH CDR3 region of a mouse/human chimeric anti-colorectal carcinoma antibody contributes hydrogen bonding to the TAG72 antigen." retrieved from STN Database accession no. 95102752 XP002134982 abstract & CANCER BIOTHERAPY, (1993 FALL) 8 (3) 253-62. , ---	23,36-47
A	WO 96 13594 A (US HEALTH) 9 May 1996 (1996-05-09) page 24, line 9 -page 26, line 3 examples 13,17,18 ---	1-47
P,A	WO 99 43816 A (ARMOUR KATHRYN ;CARR FRANK J (GB); HARRIS WILLIAM J (GB); TEMPEST) 2 September 1999 (1999-09-02) example 1 claims ---	1-47
T	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US IWAHASHI M ET AL: "CDR substitutions of a humanized monoclonal antibody (CC49): contributions of individual CDRs to antigen binding and immunogenicity." retrieved from STN Database accession no. 2000162136 XP002134983 abstract & MOLECULAR IMMUNOLOGY, (1999 OCT-NOV) 36 (15-16) 1079-91. , --- -/--	1-47

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	TAMURA M ET AL: "Structural correlates of an anticarcinoma antibody: identification of specificity-determining residues (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only." JOURNAL OF IMMUNOLOGY, (2000 FEB 1) 164 (3) 1432-41. , XP000901556 the whole document	1-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/25552

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 42 is directed to a method of treatment of the human/animal body and claims 43-47 (all partially) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/25552

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726010 A	24-07-1997	AU 706397 B	17-06-1999
		AU 1830897 A	11-08-1997
		CN 1213312 A	07-04-1999
		HU 9900396 A	28-05-1999
		NO 983284 A	16-09-1998
		PL 327929 A	04-01-1999
		US 6005091 A	21-12-1999
		ZA 9700347 A	06-10-1998
WO 9613594 A	09-05-1996	US 5889157 A	30-03-1999
		US 5981726 A	09-11-1999
		US 5608039 A	04-03-1997
		AU 4135596 A	23-05-1996
		CA 2203236 A	09-05-1996
		EP 0796334 A	24-09-1997
		JP 10508202 T	18-08-1998
		US 5990296 A	23-11-1999
WO 9943816 A	02-09-1999	AU 6439398 A	15-09-1999